

Article

Synthesis, Characterization and Biological Activity Evaluation of Some 1,N-bis-(4-amino-5-mercapto-1,2,4-triazol-3-yl) Alkanes

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Abstract: In this paper, we present the synthesis, characterization and evaluation of antiproliferative activity for four compounds carrying the 4-amino-5-mercapto-1,2,4-triazol-3-yl scaffold. The synthesis of 1,n-bis-(4-amino-5-mercapto-1,2,4-triazol-3-yl) alkanes was carried out using as starting reagents the dihydrazides of oxalic, malonic, succinic and adipic acids, using mercaptoacetic acid dianion as a leaving group, by a one-pot synthesis method implemented in our research group for the synthesis of 3-substituted-5-mercapto-1,2,4-triazoles. The compounds were obtained with modest yields (12–60%) but with good purity and were characterized by elemental analysis, FTIR, ¹H-NMR and ¹³C-NMR spectroscopy. Also, the stability of the synthesized bis-triazoles was investigated under controlled thermal stress in a dynamic oxidative atmosphere. The last part of the study consisted of biological activity evaluation, by evaluating the antiproliferative activity against the A375 line (human malignant melanoma), as well as on viability of the BJ fibroblast cell line, using MTT and LDH assays.

Keywords: 1,n-bis-(4-amino-5-mercapto-1,2,4-triazol-3-yl) alkanes; bis triazoles; anticancer activity; thermal stability; A375 line



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1. Introduction

Triazoles, particularly mercapto-substituted 1,2,4-triazoles, have become increasingly important in medicine due to their diverse biological activities [1,2], like antibacterial [3,4], antifungal [5,6], antitubercular [7], antioxidant [8], anticancer [9], anti-inflammatory [10], analgesic [11], antidiabetic [12], anticonvulsant [13], antiviral/anti-infective [14] and anxiolytic activity [15]. Numerous compounds from this class of heterocycles are promising candidates in cancer therapy, even if their exact mechanism of action is still under investigation. However, studies suggest that mercapto-substituted 1,2,4-triazoles may interfere with cancer cell growth, division and survival pathways [2].

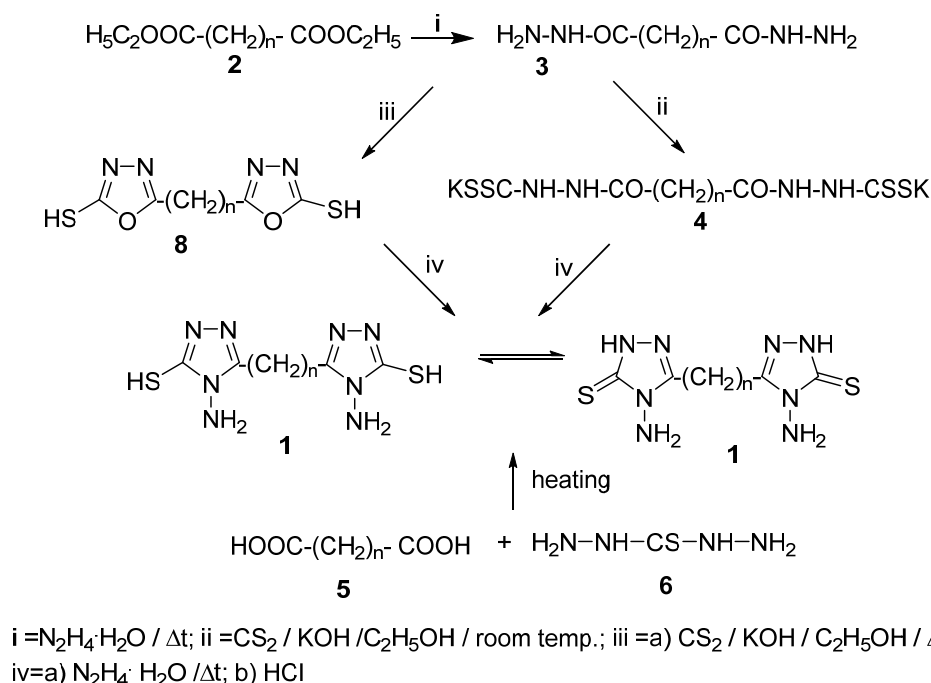
Beyond the exceptional potential of finding promising candidates from mercapto-substituted 1,2,4-triazole derivatives in oncology and cancer therapy [1,9,16], some triazole derivatives exhibit antiviral activity, making them potential candidates for treating viral, fungal and bacterial infections, with several advantages over existing drugs, like novel mechanism of action, so potentially overcoming resistance issues [17–20].

Due to the development of efficient synthetic methods for tailoring structural-diverse triazoles, these compounds are more readily available for research in the biomedical field, and some of the derivatives have been incorporated into commercially available drugs including fluconazole, voriconazole (antifungal), trazodone, nefazodone (antidepressant), trapidil (antihypertensive), estazolam (sedative-hypnotic), rufinamide (antiepileptic) [21] and taribavirin (antiviral/anti-infective) [14].

In this study, continuing our work regarding the synthesis of 3-substituted-5-mercapto-1,2,4-triazoles [4,22–26], we describe a facile synthetic pathway for a series of 1,*n*-bis-[4-amino-5-mercapto-1,2,4-triazol-3-yl] alkanes (**1**). Our preliminary attempts of obtaining 3-substituted-4-amino-5-mercapto-1,2,4-triazoles followed the synthetic reactions presented in the literature: heating 5-substituted-2-mercapto-1,3,4-oxadiazole with hydrazine hydrate in solvent, under reflux [27–33], and treating hydrazides with carbon sulfide in ethanolic KOH solution, followed by the addition of hydrazine hydrate, under reflux [34–38]. In both cases, contrary to the results presented in the literature, our synthesis reactions led to a mixture of 3-substituted-4-amino-5-mercapto-1,2,4-triazoles and 5-substituted-2-mercapto-1,3,4-oxadiazole, inseparable by recrystallization; so we adapted the synthesis method used by us previously to obtain several 1,2,4-triazoles [24,39].

Furthermore, there are studies that indicate the fact that a series of 1,*n*-bis-(4-amino-5-mercapto-1,2,4-triazol-3-yl) alkanes were found to be associated with cytotoxic [40] and pharmacological properties, such as antibacterial and anthelmintic [41,42].

Literature data report the synthesis of 1,*n*-bis-(4-amino-5-mercapto-1, 2, 4-triazol-3-yl) alkanes (**1a–d**) by the direct reaction of dicarboxylic acids (**5**), such as malonic [43], succinic [43,44], glutaric [43,44], adipic [44] and other acids with complex structures [41], mainly by melting with thiocarbohydrazide (**6**) (Scheme 1). For the 1,8-bis-(5-mercapto-4-amino-1,2,4-triazol-3-yl)-octane another synthesis reaction is reported by treating sebacic acid dihydrazide with carbon disulfide and potassium hydroxide, followed by the addition of hydrazine hydrate [45].

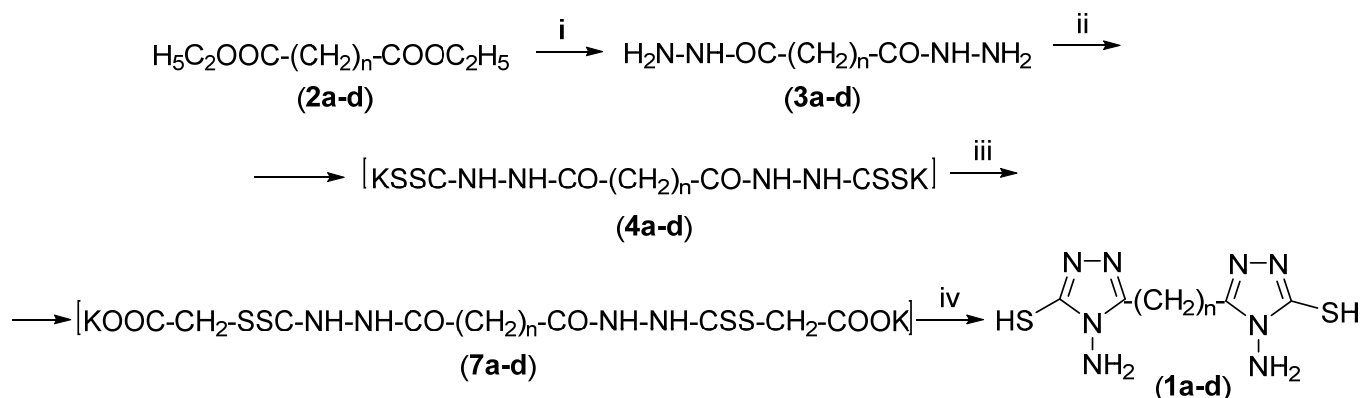


Scheme 1. Synthetic pathways for 1,*n*-bis-(4-amino-5-mercapto-1,2,4-triazol-3-yl)-alkanes (**1**).

Another method used to obtain bis-triazoles of form (**1**) consists in treating bis-oxadiazoles (**8**) with hydrazine hydrate in alcoholic medium under reflux [46].

The possible synthetic pathways for the formation of 1,*n*-bis-(5-mercapto-1,2,4-triazol-3-yl)-alkanes (**1**), with the above-mentioned properties, are shown in Scheme 1:

Our first attempts using the method from the literature [45] did not lead to obtaining bis-triazoles (**1a–d**), and following this inconvenience, we aimed towards the synthesis of these compounds by a new method, in which the hydrazides are treated with carbon sulfide, in ethanolic KOH solution, followed by the addition of potassium chloroacetate and treatment of the intermediate **7a–d** with hydrazine hydrate, under reflux. This method showed advantages in terms of overall yields, reaction time and number of steps, as well as the purity of the reaction products. According to this approach, the use of mercaptoacetic acid dianion ($^-\text{S}-\text{CH}_2-\text{COO}^-$) as a leaving group leads to pure bis-(4-amino-5-mercapto-1,2,4-triazol-3-yl)-alkanes and presents the advantage of achieving transformations **2a–d** $\rightarrow$ **3a–d** $\rightarrow$ **4a–d** $\rightarrow$ **7a–d** $\rightarrow$ **1a–d** in a single step synthesis, i.e., a “one pot reaction” (Scheme 2).



i = $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$, Δ t; ii = $\text{CS}_2/\text{KOH}/\text{EtOH}/\text{room temp.}$; iii = $\text{ClCH}_2\text{COOK}/5-10^\circ\text{C}$;
 iv = a) $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}, \Delta$ t; b) HCl ; n = 0 (**1a–4a, 7a**); 1 (**1b–4b, 7b**); 2 (**1c–4c, 7c**); 4 (**1d–4d, 7d**)

Scheme 2. Synthesis of 1,n-bis-(4-amino-5-mercapto-1,2,4-triazol-3-yl)-alkanes (**1**).

The hydrazides of oxalic, succinic, malonic and adipic acids (**3a–d**) were obtained by the hydrazinolysis of the ethyl esters of the corresponding acids (**2a–d**). All the compounds were characterized by both spectroscopic and thermal techniques. Additionally, all the synthesized compounds (**1a–d**) were screened for biological activity, namely anticancer activity against the A375 line (human malignant melanoma), as well as on viability of the BJ fibroblast cell line, using MTT and LDH assays.

2. Results and Discussion

2.1. Synthesis of Compounds

2.1.1. Synthesis and Characterization of Dihydrazides (**3a–d**) by Hydrazinolysis of Esters (**2a–d**)

In order to obtain the 4H-4-amino-5-mercapto-3-(4H-4-amino-5-mercapto-1,2,4-triazol-3-yl)-1,2,4-triazole (**1a**) and 1,n-bis-(4H-4-amino-5-mercapto-1,2,4-triazol-3-yl)alkanes (**1b–d**) derivatives, the synthesis of starting reagents was employed, namely the dihydrazides (**3a–d**) of the oxalic, malonic, succinic and adipic acids, by the hydrazinolysis of the diesters (**2a–d**).

Following the protocol presented in Section 3.2.1, the four dihydrazides were obtained with good yields (79–87%) according to the literature [47], and characterized by melting point and FTIR spectroscopy (Table 1).

Table 1. Structure and characterization of the obtained dihydrazides of oxalic, malonic, succinic and adipic acids.

Structure of Dihydrazide	Characterization of Compound		
	Molecular Formula, Molar Mass, Aspect and Synthesis Yield	Melting Point (°C) Measured/Literature	FTIR Bands (KBr, cm ⁻¹):
$\begin{array}{c} \text{CONHNH}_2 \\ \\ \text{CONHNH}_2 \end{array}$ Oxalyl dihydrazide (Oxalic acid dihydrazide) (3a)	$\text{C}_2\text{H}_6\text{N}_4\text{O}_2$ $M = 118 \text{ g}\cdot\text{mol}^{-1}$ White powder yield $\eta = 82\%$	190–194/ 242–244 [48]	3286, 3195, 1650, 1612, 1504, 748
$\begin{array}{c} \text{CONHNH}_2 \\ \\ \text{CH}_2 \\ \\ \text{CONHNH}_2 \end{array}$ Malonic dihydrazide (Malonic acid dihydrazide) (3b)	$\text{C}_3\text{H}_8\text{N}_4\text{O}_2$ $M = 132 \text{ g}\cdot\text{mol}^{-1}$ White powder yield $\eta = 79\%$	152–157/ 152–154 [49]	3330, 3035, 1626; 1076; 1122; 1504
$\begin{array}{c} \text{CONHNH}_2 \\ \\ (\text{CH}_2)_2 \\ \\ \text{CONHNH}_2 \end{array}$ Succinic dihydrazide (Succinic acid dihydrazide) (3c)	$\text{C}_4\text{H}_{10}\text{N}_4\text{O}_2$ $M = 146 \text{ g}\cdot\text{mol}^{-1}$ White powder yield $\eta = 84\%$	166–171/ 170–171 [50]	3289; 3195; 1540; 1509
$\begin{array}{c} \text{CONHNH}_2 \\ \\ (\text{CH}_2)_4 \\ \\ \text{CONHNH}_2 \end{array}$ Adipic dihydrazide (Adipic acid dihydrazide) (3d)	$\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2$ $M = 174 \text{ g}\cdot\text{mol}^{-1}$ White powder yield $\eta = 87\%$	174–181/ 180–182 [51]	3200, 3311, 3181, 3047, 2925; 1636, 1536

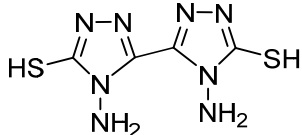
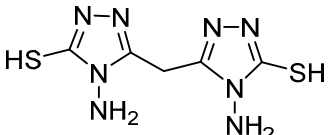
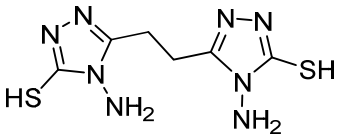
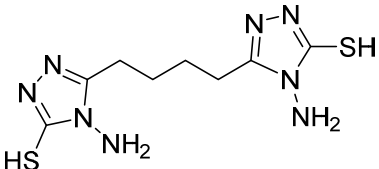
The measured melting points are in good agreement with literature data for the **3b–d** derivatives, while for **3a** there is a considerable difference, which can be explained by the fact that this compound is known to exist in at least four polymorphic states [52]. Following this aspect, the existence of several solid forms of organization of this compound determines different physical properties (including melting point), since the formation of hydrogen bonds between donor and acceptor moieties in this molecule is conditioned by the different orientations of the groups with respect to the molecular plane, which are different geometric orientations for hydrogen bonding arrangements in the solid state [52,53].

2.1.2. Synthesis and Characterization of Bis-Triazoles **1a–d**

Synthesis of 4*H*-4-amino-5-mercapto-3-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)-1,2,4-triazole (**1a**) and 1,*n*-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)alkanes (**1b–d**) started from dihydrazides (**3a–d**), as presented in Scheme 2.

Following the protocol presented in Section 3.2.2, the four triazoles were obtained with variable yields (12–60%), and characterized by melting point, elemental analysis, FTIR, ¹H-NMR and ¹³C-NMR spectroscopy (Table 2).

Table 2. Structure and characterization of the obtained triazoles.

Structure of Triazole	Molecular Formula, Molar Mass, Aspect and Synthesis Yield	Melting Point (°C)	FTIR Bands (KBr, cm ⁻¹):	Characterization of Compound	
				Elemental Analysis % (Calcd./Found)	
				¹ H-NMR δ _H (DMSO- <i>d</i> ₆ , 200 MHz)	¹³ C-NMR δ _C (DMSO- <i>d</i> ₆ , 50 MHz)
 4-amino-5-mercapto-3-(4 <i>H</i> -4-amino-5-mercapto-1,2,4-triazol-3-yl)-1,2,4-triazole (1a)	C ₄ H ₆ N ₈ S ₂ M = 230.72 g·mol ⁻¹ White powder yield η = 33%	>350 * (201 °C)	3330; 3044; 1590; 1508; 1449; 1251; 991	C-20.86; H-2.63; N-48.66/ C-20.49; H-2.48; N-48.14 11,96 (s, 2H, NH); 5,92 (s, 4H, NH ₂)	167,07 (3-C); 139,30 (5-C);
 1,1-bis-(4 <i>H</i> -4-amino-5-mercapto-1,2,4-triazol-3-yl)methane (1b)	C ₅ H ₈ N ₈ S ₂ , M = 244.30 g·mol ⁻¹ White powder yield η = 12%	281–282 * (181 °C)	3291; 3242; 3076; 3026; 2923; 2807; 1614; 1572; 1235; 980	C-24.58; H-3.30; N-45.87/ C-24.11; H-3.18; N-45.26 13,39 (s, 1H, NH); 5,51 (s, 2H, -NH ₂) 4,14 (s, 2H, -CH ₂ -);	166,19 (3-C); 147,15 (5-C); 21,50 (-CH ₂ -)
 1,2-bis-(4 <i>H</i> -4-amino-5-mercapto-1,2,4-triazol-3-yl)ethane (1c)	C ₆ H ₁₀ N ₈ S ₂ , M = 258.32 g·mol ⁻¹ White powder yield η = 12%	252–254 *	3287; 3159; 2941; 2755; 1615; 1561; 1477; 969	C-27.90; H-3.90; N-43.38/ C-27.77; H-3.84; N-43.02 13,33 (s, 1H, NH); 5,54 (s, 2H, -NH ₂); 3,07 (s, 2H, -CH ₂ -)	165,86 (3-C); 150,68 (5-C); 20,79 (-CH ₂ -)
 1,4-bis-(4 <i>H</i> -4-amino-5-mercapto-1,2,4-triazol-5-yl)butane (1d)	C ₈ H ₁₄ N ₈ S ₂ , M = 286.38 g·mol ⁻¹ White powder yield η = 60%	252–253 *	3284; 3135; 3043; 2951; 2771; 1615; 1562; 1488; 1296; 743	C-33.55; H-4.93; N-39.13/ C-33.18; H-4.74; N-38.94 13,25 (s, 2H, NH); 5,51 (s, 4H, NH ₂); 2,60–2,67 (m, 2H, Tz-CH ₂ -CH ₂ -); 1,54–1,70 (m, 2H, Tz-CH ₂ -CH ₂ -)	165,63 (3-C); 151,89 (5-C); 24,97 (Tz-CH ₂ -CH ₂ -); 23,68 (Tz-CH ₂ -CH ₂ -);

* melting with decomposition (browning temperature).

The analysis of the FTIR spectra of 4-amino-5-mercapto-3-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)-1,2,4-triazole (**1a**) reveals the appearance of several characteristic bands [40]. For the primary amino group, the N–H stretching vibration is revealed by the band with a maximum at 3330 cm⁻¹. The stretching vibrations of the aromatic C–H bond at 3044 cm⁻¹, as well as the stretching vibrations of the double C=N bonds, can be identified in the spectrum at 1590 cm⁻¹. The existence of the compound in the thione tautomeric form (C=S) is confirmed by the band that appears at 1251 cm⁻¹ [40,54].

The presence of the primary amino groups (–NH₂) in the structure of 1,1-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)methane (**1b**) is indicated by the presence of the bands at 3291 and 3242 cm⁻¹. The stretching vibrations of the aromatic C–H bond are observed at 3076 and 3026 cm⁻¹, as well as the symmetric and asymmetric vibrations corresponding to the CH₂ group at 2923 and 2807 cm⁻¹. The stretching vibrations of the C=N bonds are represented by the band observed at 1614, while the C=S vibration is represented by a band at 1235 cm⁻¹.

The IR spectrum of 1,2-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)ethane (**1c**) shows an absorption band at 3287 cm⁻¹, indicating the presence of the primary amino group

(-NH_2) in the molecule. The stretching vibrations of the aromatic C–H bonds are observed at 3159 cm^{-1} , while the stretching vibrations of the C=N and C=S bonds appear at 1615 and 1248 cm^{-1} , respectively, as medium-intense bands. The symmetric and asymmetric stretching vibrations for the CH_2 groups are represented by the bands seen at 2941 and 2755 cm^{-1} . In the case of 1,4-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-5-yl)butane (**1d**), the stretching vibrations (N–H) for the amino group are observed at 3284 cm^{-1} , while the bands at 3135 and 3043 cm^{-1} characterize the stretching vibrations of the aromatic C–H bond. The bands at 2951 and 2771 cm^{-1} can be assigned to the symmetric and asymmetric vibrations of the CH_2 groups, and the stretching vibrations of the C=N and C=S bonds can be seen at 1615 and 1235 cm^{-1} as medium-intense bands.

The ^{13}C -NMR spectra of compounds **1a–d** highlights the presence of only the tautomeric form (C=S) through the signals $\delta = 165.63\text{--}167.07\text{ ppm}$, corresponding to the C=S exocyclic bonds. The ^1H -NMR spectra of compounds **1a–d** reveal a singlet in $\delta = 11.96\text{--}13.30\text{ ppm}$ region due to the triazole protons (NH), confirming the closure of the triazole rings. The protons of the NH_2 groups appear between $\delta = 5.51\text{--}5.92\text{ ppm}$, demonstrating the presence of the free amino groups on the triazole ring, while the signals from the alkyl chain of compounds **1b–d** are observed at $\delta = 1.70\text{--}4.14\text{ ppm}$. As further proof, FTIR, NMR 2D COSY ^1H - ^{13}C , ^{13}C -NMR DEPT-135 and 2D HMBC spectra were recorded, as seen in Supplementary Material, Figures S1–S18.

Also, the other signals observed in both the ^{13}C -NMR and ^1H -NMR spectra are presented in Table 2, confirming the identity, structure and purity of the synthesized compounds.

2.2. Thermal Analysis Investigations

The obtained thermoanalytical curves TG/DTG for compounds **1a–d** are presented in Figure 1A–D.

The thermal treatment of compound **1a** suggests that it is thermally stable up to $194\text{ }^\circ\text{C}$, when the first mass loss occurs up to $284\text{ }^\circ\text{C}$ (DTG first process between $194\text{--}246\text{ }^\circ\text{C}$, DTG_{peak} at $204\text{ }^\circ\text{C}$; DTG second process between $246\text{--}284\text{ }^\circ\text{C}$, DTG_{peak} at $267\text{ }^\circ\text{C}$), with a corresponding mass loss $\Delta m = 15.06\%$. The main mass loss due to thermolysis takes place in the $284\text{--}597\text{ }^\circ\text{C}$ temperature range (DTG third process between $303\text{--}597\text{ }^\circ\text{C}$, DTG_{peak} at $425\text{ }^\circ\text{C}$), the residual mass being insignificant (less than 1%). Surprisingly, the behavior observed under thermal stress is different when the heating takes place in the furnace of the Setline instrument vs. Optimelt apparatus, a fact that can be explained by the different environments around the solid sample under heating: dynamic air/oxidative atmosphere with considerable flow, vs. static air atmosphere in the capillary glass. However, the browning observed at $201\text{ }^\circ\text{C}$ during the melting point investigation can be associated with the first mass loss process from the TG/DTG curve (Figure 1A).

Compound **1b** has good thermal stability up to $181\text{ }^\circ\text{C}$ when a small mass loss ($\Delta m = 0.19\%$) takes place up to $232\text{ }^\circ\text{C}$ (DTG first process between $181\text{--}232\text{ }^\circ\text{C}$, DTG_{peak} at $197\text{ }^\circ\text{C}$); in the $232\text{--}309\text{ }^\circ\text{C}$ temperature range, the first considerable mass loss takes place ($\Delta m = 19.38\%$, DTG process between $232\text{--}308\text{ }^\circ\text{C}$, DTG_{peaks} at 258 and $285\text{ }^\circ\text{C}$, respectively), followed by another considerable mass loss process in the $309\text{--}624\text{ }^\circ\text{C}$ temperature range ($\Delta m = 80.41\%$, DTG_{peaks} at 396 and $501\text{ }^\circ\text{C}$, respectively).

Thermal analysis of compound **1c** reveals that is thermally stable up to $238\text{ }^\circ\text{C}$ when a multistep mass loss takes place, according to the DTG curve; the first mass loss process occurs in a narrow temperature range ($238\text{--}266\text{ }^\circ\text{C}$, $\Delta m = 14.44\%$, DTG_{peak} at $252\text{ }^\circ\text{C}$ —in agreement with the melting indicated by the Optimelt apparatus), followed by several other processes, up to $678\text{ }^\circ\text{C}$ ($\Delta m = 83.4\%$, DTG_{peaks} at 287 , 315 and $647\text{ }^\circ\text{C}$).

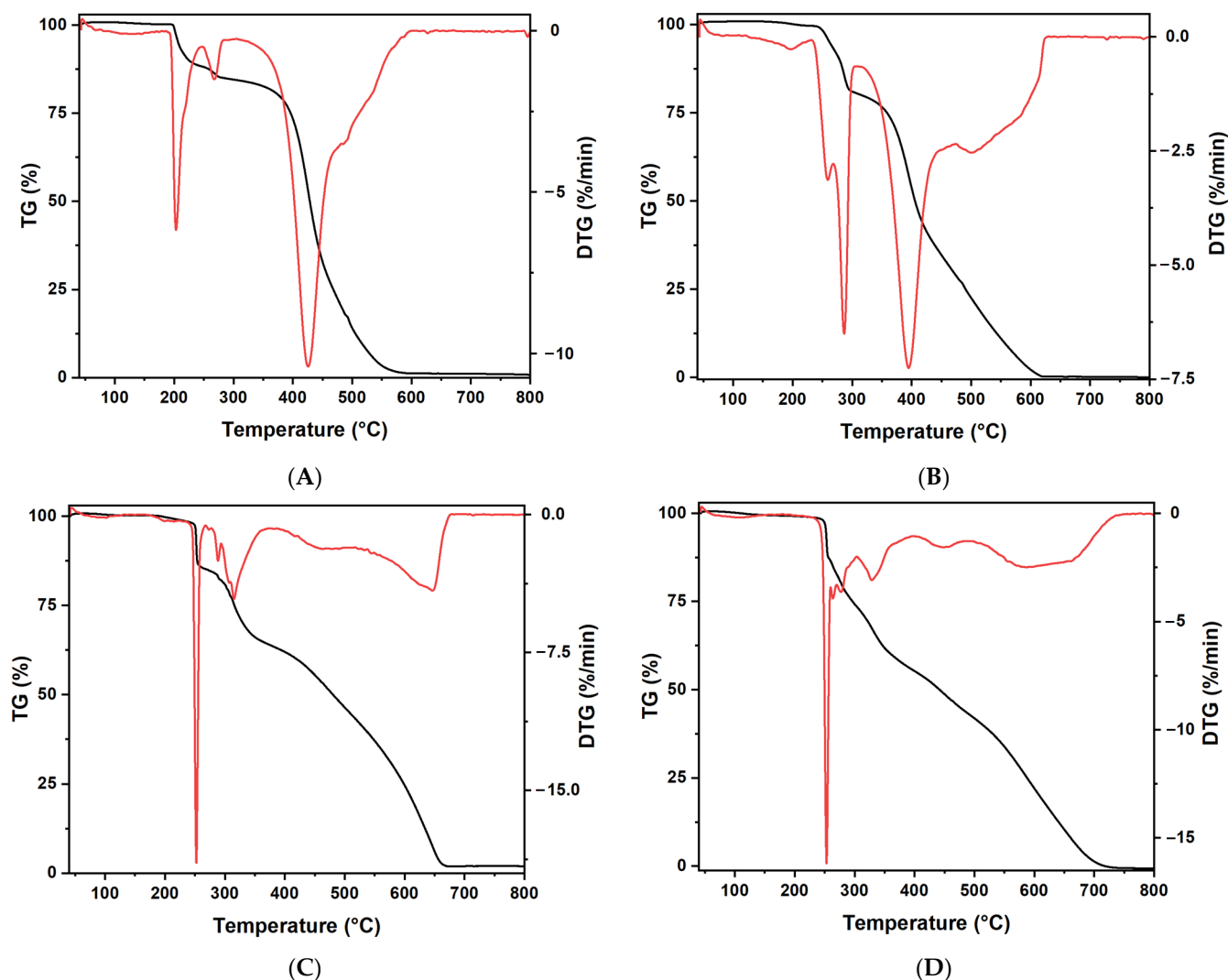


Figure 1. TG (red line)/DTG (black line) data recorded for synthesized triazoles, namely **1a** (A), **1b** (B), **1c** (C) and **1d** (D) in a dynamic air atmosphere at $\beta = 10\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$.

Similar thermal behavior to that observed for the **4c** derivative is observed for the **1d** compound. This triazole is stable up to $232\text{ }^{\circ}\text{C}$, when a multistep mass loss takes place, according to the DTG curve; the first mass loss process occurs in a narrow temperature range ($238\text{--}261\text{ }^{\circ}\text{C}$, $\Delta m = 13.64\%$, DTG_{peak} at $252\text{ }^{\circ}\text{C}$ —in agreement with the melting indicated by the Optimelt apparatus), followed by several other processes, up to $750\text{ }^{\circ}\text{C}$ ($\Delta m = 86.34\%$, $\text{DTG}_{\text{peaks}}$ at $264, 276, 330, 448$ and $583\text{ }^{\circ}\text{C}$).

2.3. Antiproliferative Activity

The compounds showed different activity against human cancer and normal cells at $10\text{ }\mu\text{M}$ and $30\text{ }\mu\text{M}$ concentrations. The analysis of activity on the cancer cell line showed a proportional decrease in viability in rapport with drug concentration, in most cases. Overall, cellular activity was lower than the control group. The untreated control group is considered as 100% mitochondrial viability as they represent cells with no treatment applied. A375 cells treated with **1a**, **1b**, **1c**, or **1d** at a $10\text{ }\mu\text{M}$ concentration expressed a viability of $88.94 (\pm 1.64)\%$, $118.00 (\pm 2.94)\%$, $100.76 (\pm 2.80)\%$, or $99.98 (\pm 6.71)\%$, respectively. A375 cells treated with **1a**, **1b**, **1c**, or **1d** at a $30\text{ }\mu\text{M}$ concentration expressed a viability of $98.63 (\pm 5.09)\%$, $92.49 (\pm 10.46)\%$, $96.61 (\pm 8.25)\%$, or $94.30 (\pm 6.88)\%$, respectively. Compound **1a** decreased viability by 9.69% for the $10\text{ }\mu\text{M}$ concentration compared to the $30\text{ }\mu\text{M}$ concentration. For the rest of the compounds, viability was decreased by 4.62%, 4.15%, or

5.67% more for the 30 μ M concentration than the 10 μ M concentration (Figure 2). Fibroblast analysis showed an increase in viability of 17–42% for a 10 μ M concentration and 28–81% for a 30 μ M concentration compared to the control group. Fibroblast analysis showed an increase in viability for most of the compounds tested. BJ cells treated with **1a**, **1b**, **1c**, or **1d** at a 10 μ M concentration expressed a viability of 117.96 (± 7.81)%, 101.03 (± 16.01)%, 177.09 (± 17.66)%, or 148.61 (± 11.45)%, respectively. BJ cells treated with **1a**, **1b**, **1c**, or **1d** at a 30 μ M concentration expressed a viability of 168.67 (± 16.16)%, 96.41 (± 6.04)%, 196.90 (± 21.07)%, or 122.14 (± 4.12)%, respectively. Compounds **1a** and **1c** increased viability by 50.71% and 19.81%, respectively, for the 30 μ M concentration compared to the 10 μ M concentration. Compounds **1b** and **1d** increased viability by 4.61% and 26.47%, respectively for the 10 μ M concentration compared to the 30 μ M concentration (Figure 3).

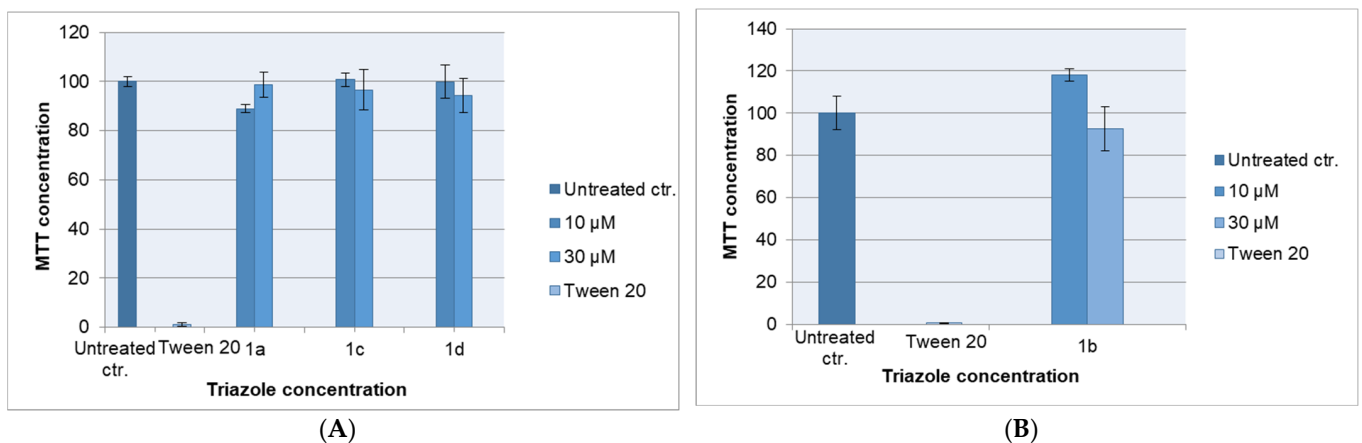


Figure 2. The results of the MTT assay for A375 cell line for compounds **1a**, **1c** and **1d** (A) and **1b**, respectively (B).

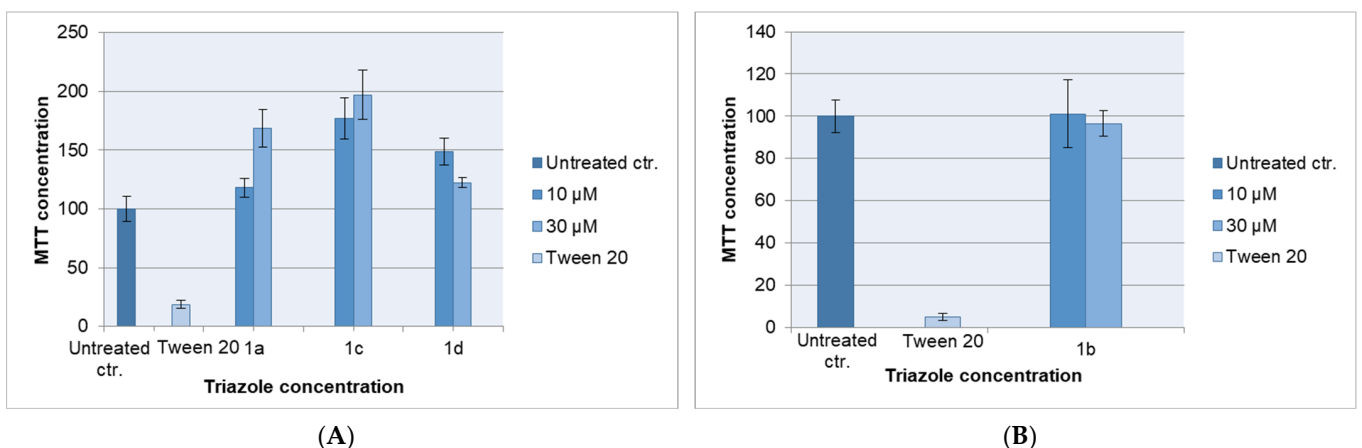


Figure 3. The results of the MTT assay for BJ cell line for compounds **1a**, **1c** and **1d** (A) and **1b**, respectively (B).

LDH assay results showed a decrease in cancer cell growth for the treated group, which resulted in lower LDH release compared with untreated control. Untreated cancer cells released 0.117 (± 0.0059) U/mL LDH. A375 cells treated with **1a**, **1c**, or **1d** at a 10 μ M concentration released 0.106 (± 0.0016) U/mL, 0.108 (± 0.0026) U/mL, or 0.106 (± 0.0062) U/mL LDH, respectively. A375 cells treated with **1a**, **1c**, or **1d** at a 10 μ M concentration released 0.109 (± 0.0029) U/mL, 0.107 (± 0.0068) U/mL, or 0.108 (± 0.0026) U/mL LDH, respectively. Compound **1b** was analyzed in another plate and had an untreated control value of 0.0584 U/mL and released 0.0497 U/mL. Compounds **1a**, **1b**, and **1d** decreased LDH release by 0.0026 U/mL, 0.00087 U/mL, or 0.0021 U/mL, respectively for the 10 μ M

concentration compared to the 30 μM concentration, while **1c** decreased LDH release by 0.0008 U/mL for the 30 μM concentration compared to the 10 μM concentration. (Figure 4). Data showed a slight cell growth inhibition for treated groups, but higher compound concentration had an insignificant impact compared to a lower dose.

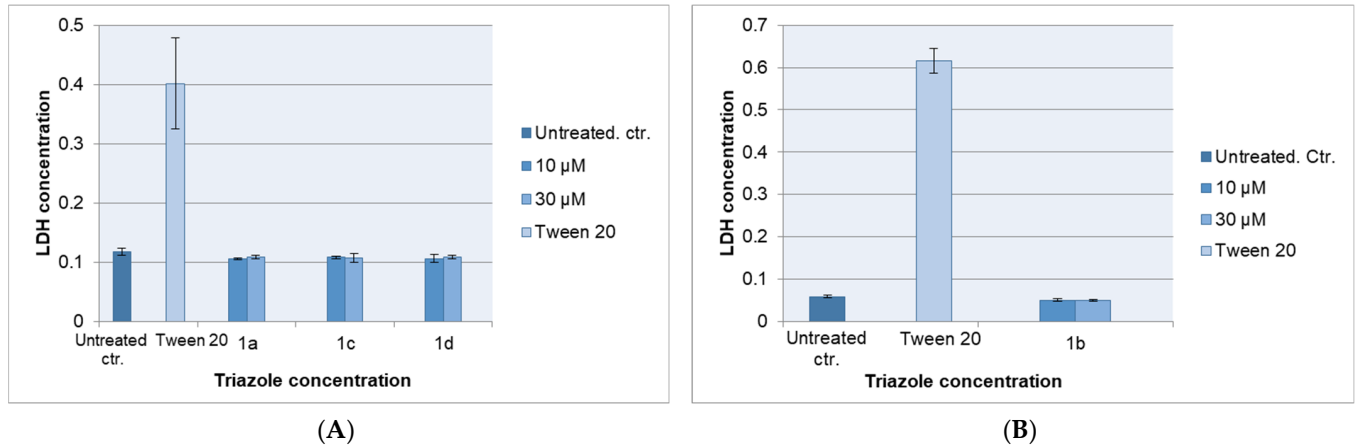


Figure 4. The results of the LDH assay for A375 cell line for compounds **1a**, **1c** and **1d** (A) and **1b**, respectively (B).

Also, fibroblast showed little to no changes in LDH concentration. BJ cells had an untreated control value of 0.103 (± 0.010) U/mL. BJ cells treated with **1a**, **1c**, or **1d** at a 10 μM concentration released 0.104 (± 0.003) U/mL, 0.098 (± 0.006) U/mL, or 0.114 (± 0.007) U/mL, respectively. BJ cells treated with **1a**, **1c**, or **1d** at a 30 μM concentration released 0.094 (± 0.007) U/mL, 0.101 (± 0.007) U/mL, or 0.122 (± 0.008) U/mL, respectively. Compound **1b** was analyzed in another plate and had an untreated control value of 0.146 (± 0.0068) U/mL and released 0.110 (± 0.0028) U/mL at a 10 μM concentration and 0.109 (± 0.0055) U/mL at a 30 μM concentration. Compound **1a** at 10 μM and **1d** at 10 μM and 30 μM increased LDH release by 0.001 U/mL, 0.011 U/mL and 0.0019 U/mL, respectively, compared with the control group. Compounds **1c** and **1b** at 10 μM and 30 μM decreased LDH release by 0.004 U/mL and 0.002 U/mL and 0.036 U/mL and 0.037 U/mL, respectively compared with the control group (Figure 5).

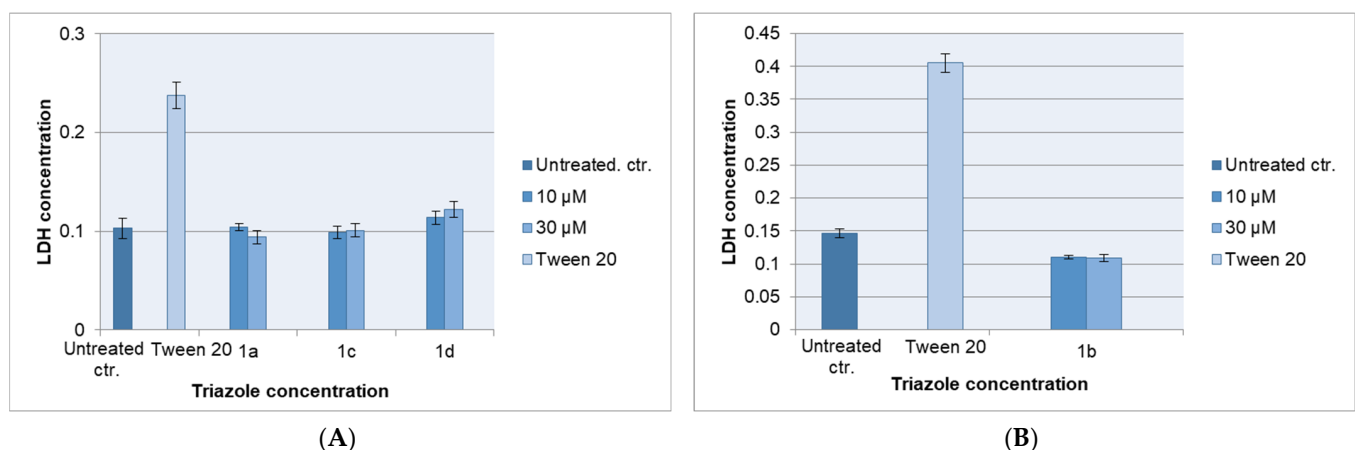


Figure 5. The results of the LDH assay for BJ cell line for compounds **1a**, **1c** and **1d** (A) and **1b**, respectively (B).

3. Materials and Methods

3.1. Reagents and Instrumentation

The reagents were commercial products of analytical purity from Chimopar (București, Romania), Merck (Darmstadt, Germany), Fluka-Honeywell (Charlotte, NC, USA) and used as received. The synthesis of ethyl esters of oxalic, malonic, succinic and adipic acids (**2a–d**), was performed according to the indications mentioned in the literature.

Melting points were determined using an Optimelt M100 (Stanford Research Device, Stanford Research Systems, Inc., Sunnyvale, CA, USA) instrument in open capillary tubes, in the heating range 30–350 °C, using a heating rate of 10 °C·min^{−1} (the values are uncorrected).

Thin-layer chromatography (TLC) was carried out on silica-gel-coated plates 60 F254 Merck using benzene:methanol 7:3 (*v/v*) as eluant. The chromatographic spots were revealed by exposure to iodine vapors and/or UV light irradiation ($\lambda = 254$ nm).

The percentage of C, H and N were obtained by means of elemental analysis using a Vario El Cube apparatus (Elementar Analysensysteme GmbH, Hanau, Germany).

FTIR spectra were recorded in KBr pellets using a Specac Pellet Press (Specac Ltd., Kent, UK) using a Jasco FT/IR-410 spectrophotometer (Jasco Analytical Instruments, Easton, PA, USA).

¹H-NMR and ¹³C-NMR spectra were recorded using a Bruker Avance AC200 spectrometer (Bruker Biospin International, Ag, Aegeristrasse, Switzerland) in DMSO-*d*₆, using TMS as reference; chemical shifts are reported in ppm and the coupling constants in Hz. The multiplicity of the signal and the aspect of the band are abbreviated as follows: br: broad; s: singlet; d: doublet; m: multiplet.

Thermoanalytical curves were obtained using a Setline TGA (SETARAM, Caluire, France) instrument. TG (thermogravimetric/mass curve) and DTG data (derivative thermogravimetric/mass derivative) were recorded on samples placed in an open alumina crucible. The heating process was conducted in dynamic airflow (100 mL·min^{−1}) under non-isothermal conditions at a heating rate $\beta = 10$ °C·min^{−1}, from a temperature of 40 °C up to 800 °C, each sample having a mass of approximately 10 mg. Each TG experiment was performed in duplicate and the results were practically identical.

3.2. Synthesis Protocols

3.2.1. Synthesis of Dihydrazides (**3a–d**) by Hydrazinolysis of Esters (**2a–d**)

The ethyl ester of each dicarboxylic acid (0.1 mol) was dissolved in absolute ethanol (10 mL), then hydrazine hydrate (0.3 mol) was added and heated under reflux for 8–10 h. After dry distillation of the reaction mixture, at 80–90 °C/5mmHg, the solid residue was crystallized from ethanol.

3.2.2. Synthesis of 4*H*-4-amino-5-mercapto-3-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)-1,2,4-triazole (**1a**) and 1, *N*-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)alkanes (**1b–d**)

The dihydrazide of dicarboxylic acid (0.02 mol) was dissolved in an ethanolic potassium hydroxide solution (0.06 mol KOH/40 mL of EtOH), then carbon sulfide (0.06 mole) was added and stirred at room temperature for two hours. The reaction mixture was cooled at 5–10 °C in an ice bath and then, an aqueous potassium chloroacetate solution (0.04 mole ClCH₂COOK/30 mL H₂O) was added. After 45 min, hydrazine hydrate (0.04 mol) was added at room temperature. After two hours, half of the reaction volume was distilled under vacuum, followed by the addition of hydrazine hydrate (0.04 mol), and left under reflux for 4 h. The solution was cooled to room temperature, brought to pH~1 with concentrated HCl, and the products were crystallized from aqueous ethanol solution.

3.3. Cell Culture

The cells were cultivated in Dulbecco's Modified Eagle's Medium (DMEM), a medium containing high glucose, supplemented with 10% fetal bovine serum (FBS), 1% Penicillin/Streptomycin, 1% L-glutamate and 1% essential amino acids.

The cellular death and viability experiments were conducted using cells purchased from ATCC, namely, the human melanoma (A375) (ATCC CRL-1619) and fibroblast (BJ) (ATCC CRL-2522) cell lines. The stock culture was preserved at a temperature of -80°C in a medium containing dimethylsulfoxide (DMSO). The unfrozen cells were maintained in an incubator in a temperature of 37°C , a 5% concentration of CO_2 , and a humidified atmosphere.

Assays were conducted roughly after approximately 80% of the bottom flask was populated with cells. The cells were cultivated in a 96-well microplate with a concentration of 104 cells per well for the subsequent analysis. The cells were incubated for 24 h after seeding, allowing them to attach to the well bottom. The treatment was administered at concentrations of 10 μM and 30 μM . Each concentration had 8 replicas for each substance used, besides the control group for each microplate. The positive control consisted of cells without any treatment, while the negative control consisted of cells treated with 10 μL /well of Tween 20. The flask containing the treatment was incubated for a further 24 h, and the results were analyzed using LDH and MTT assays.

3.4. Cellular Viability Assay

The proliferation was evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution, at a concentration of 0.5 mg/mL, which was added in a concentration of 100 μL /well, after the media was removed, and incubated for 2 h. Tetrazolium salt enters cell membranes and undergoes reduction by mitochondrial and cytosolic enzymes, resulting in the formation of purple formazan crystals. After incubation, MTT solution was removed by pipetting, and formazan crystals were dissolved by adding 100 μL isopropanol to each well. The microplate was analyzed using a spectrophotometer at a wavelength of 550 nm, with a reference wavelength of 630 nm to eliminate cellular debris errors.

The viability percentage was calculated by multiplying the optical density at 570 nm of the experimental sample by 100 and dividing it by the optical density at 570 nm of the control sample.

Viability assay by LDH release measures the level of plasma membrane damage. LDH oxidizes L-lactate to pyruvate and the reaction is mediated by the hydrogen acceptor, Nicotinamide Adenine Dinucleotide (NAD^+). The process produces NADH, which then will reduce a yellow tetrazolium salt called iodonitrotetrazolium (INT). A total of 50 μL of culture medium from the treated microplate was transferred to another microplate, onto which reagents were then added. Reagent concentrations were 22.2 mg/mL TRIS, 19.6 mg/mL Li-Lactate and 3.44 mg/mL NAD. The reagent solution in each well consisted of 50 μL of TRIS, 50 μL Li-Lactate and 50 μL NAD. The microplate was placed in a microplate reader after 5 min, and the absorbance was measured at 490 nm using a reference wavelength of 630 nm to eliminate errors caused by cellular debris.

The unit of measurement [U/mL] was calculated using the formula $(A \cdot V_t)/(\epsilon \cdot d \cdot V_p)$, where: Absorbance is multiplied by the total volume of reagents in the well, which is up to 0.2 mL. Millimolar extinction coefficient (ϵ) of INT to formazan at a wavelength of 490 nm is $18.4 \text{ mM}^{-1}\text{cm}^{-1}$. The reading light path (d) of the reaction is determined by the diameter of the 96-well plate, which is 6.4 mm, resulting in an area of 0.32 cm^2 . The volume of the reaction liquid is 0.2 cm^3 , so the light path is calculated as 0.2 cm^3 divided by 0.32 cm^2 , resulting in a light path of 0.625 cm. V_p represents the volume of the sample that is being examined for the reaction, which is equal to 50 microliters or 0.05 milliliters.

4. Conclusions

Four compounds carrying the 4-amino-5-mercapto-1,2,4-triazol-3-yl moiety were synthesized, characterized and evaluated for their biological activity. The synthesis of 1,n-bis-(4-amino-5-mercapto-1,2,4-triazol-3-yl) alkanes was carried out by a new method, previously employed in our research group for the synthesis of 3-substituted-4-amino-5-mercapto-1,2,4-triazoles. In this study, simple and highly accessible starting reagents were used, namely, the dihydrazides of oxalic, malonic, succinic and adipic acids, using mercap-

toacetic acid dianion as a leaving group. Initially, the compounds were characterized by elemental analysis, which confirmed the theoretical composition, and by FTIR spectroscopy, which revealed the existence of characteristic bands of the moieties from bis-triazole structures. Although the compounds were obtained with modest yields (12–60%), their purity was high.

Later, ^1H -NMR and ^{13}C -NMR spectroscopy were employed, the spectral results confirming the identity and purity of the designed compounds. The last part of our study aimed toward determining the stability profile of these compounds under controlled thermal stress in oxidative atmosphere. The biological activity evaluation consisted of studying the anticancer activity against the A375 line (human malignant melanoma), as well as on viability of the BJ fibroblast cell line, using MTT and LDH assays.

As future directions of research, we aim toward optimization of the reaction yields and extend the homolog series of the bis-triazoles by using long-chained dicarboxylic acids. Also, a study for the reaction mechanism of this synthetic protocol will be investigated.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app14146180/s1>. Figure S1. FT-IR spectra of 1a, 4-amino-5-mercapto-3-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)-1,2,4-triazole; Figure S2. ^1H -NMR spectra of 1a, 4-amino-5-mercapto-3-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)-1,2,4-triazole; Figure S3. ^{13}C -NMR spectra of 1a, 4-amino-5-mercapto-3-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)-1,2,4-triazole; Figure S4. 2D COSY ^1H - ^{13}C spectra of 1a, 4-amino-5-mercapto-3-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)-1,2,4-triazole; Figure S5. ^{13}C -RMN DEPT-135 spectra of 1a, 4-amino-5-mercapto-3-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)-1,2,4-triazole; Figure S6. 2D HMBC NMR spectra of 1a, 4-amino-5-mercapto-3-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)-1,2,4-triazole; Figure S7. FT-IR spectra of 1b, 1,1-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)methane; Figure S8. ^1H -NMR spectra of 1b, 1,1-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)methane; Figure S9. ^{13}C -NMR spectra of 1b, 1,1-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)methane; Figure S10. FT-IR spectra of 1c, 1,2-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)ethane; Figure S11. ^1H -NMR spectra of 1c, 1,2-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)ethane; Figure S12. ^{13}C -NMR spectra of 1c, 1,2-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-3-yl)ethane; Figure S13. FT-IR spectra of 1d, 1,4-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-5yl)butane; Figure S14. ^1H -NMR spectra of 1d, 1,4-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-5yl)butane; Figure S15. ^{13}C -NMR spectra of 1d, 1,4-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-5yl)butane; Figure S16. 2D COSY ^1H - ^{13}C spectra of 1d, 1,4-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-5yl)butane; Figure S17. ^{13}C -RMN DEPT-135 spectra of 1d, 1,4-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-5yl)butane; Figure S18. 2D HMBC NMR spectra of 1d, 1,4-bis-(4*H*-4-amino-5-mercapto-1,2,4-triazol-5yl)butane.

Author Contributions: Conceptualization, A.-A.A., V.-N.B., A.L. and I.L.; Data curation, A.L., V.B., A.R. and M.M.; Formal analysis, A.R. and M.M.; Investigation, A.-A.A., V.-N.B., S.E.B., A.L., V.B., A.R. and I.L.; Methodology, A.-A.A., V.-N.B., S.E.B., A.L. and V.B.; Resources, S.E.B.; Software, A.L. and V.B.; Supervision, V.-N.B. and I.L.; Validation, V.-N.B. and A.L.; Visualization, M.M.; Writing—original draft, A.-A.A., V.-N.B., S.E.B., A.L., A.R. and I.L.; Writing—review and editing, A.-A.A., V.-N.B., A.L., M.M. and I.L. Synthesis was performed by A.-A.A. and V.-N.B.; Structural Characterization and Physiochemical Evaluations were performed by A.L., V.B., A.R. and I.L.; Biological Assays were performed by S.E.B. and M.M. All authors have read and agreed to the published version of the manuscript.

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References

1. Matin, M.M.; Matin, P.; Rahman, M.R.; Ben Hadda, T.; Almalki, F.A.; Mahmud, S.; Ghoneim, M.M.; Alruwaily, M.; Alshehri, S. Triazoles and Their Derivatives: Chemistry, Synthesis, and Therapeutic Applications. *Front. Mol. Biosci.* **2022**, *9*, 864286. [[CrossRef](#)] [[PubMed](#)]
2. Aggarwal, R.; Sumran, G. An insight on medicinal attributes of 1,2,4-triazoles. *Eur. J. Med. Chem.* **2020**, *205*, 112652. [[CrossRef](#)] [[PubMed](#)]
3. Strzelecka, M.; Świątek, P. 1,2,4-Triazoles As Important Antibacterial Agents. *Pharmaceuticals* **2021**, *14*, 224. [[CrossRef](#)] [[PubMed](#)]
4. Ledeti, I.; Bercean, V.; Alexa, A.; Șoica, C.; Șuta, L.M.; Dehelean, C.; Trandafirescu, C.; Muntean, D.; Licker, M.; Fuliș, A. Preparation and antibacterial properties of substituted 1,2,4-triazoles. *J. Chem.* **2015**, *2015*, 879343. [[CrossRef](#)]
5. Appna, N.R.; Nagiri, R.K.; Korupolu, R.B.; Kanugala, S.; Chityal, G.K.; Thipparapu, G.; Banda, N. Design and synthesis of novel 4-hydrazone functionalized/1,2,4-triazole fused pyrido[2,3-d]pyrimidine derivatives, their evaluation for antifungal activity and docking studies. *Med. Chem. Res.* **2019**, *28*, 1509–1528. [[CrossRef](#)]
6. Karaca Gençer, H.; Acar Çevik, U.; Levent, S.; Sağlık, B.N.; Korkut, B.; Özkay, Y.; İlgin, S.; Öztürk, Y. New Benzimidazole-1,2,4-Triazole Hybrid Compounds: Synthesis, Anticandidal Activity and Cytotoxicity Evaluation. *Molecules* **2017**, *22*, 507. [[CrossRef](#)] [[PubMed](#)]
7. Rode, N.D.; Sonawane, A.D.; Nawale, L.; Khedkar, V.M.; Joshi, R.A.; Likhite, A.P.; Sarkar, D.; Joshi, R.R. Synthesis, biological evaluation, and molecular docking studies of novel 3-aryl-5-(alkyl-thio)-1H-1,2,4-triazoles derivatives targeting Mycobacterium tuberculosis. *Chem. Biol. Drug Des.* **2017**, *90*, 1206–1214. [[CrossRef](#)] [[PubMed](#)]
8. Peng, Z.; Wang, G.; Zeng, Q.H.; Li, Y.; Wu, Y.; Liu, H.; Wang, J.J.; Zhao, Y. Synthesis, antioxidant and anti-tyrosinase activity of 1,2,4-triazole hydrazones as antibrowning agents. *Food Chem.* **2021**, *341*, 128265. [[CrossRef](#)] [[PubMed](#)]
9. Grytsai, O.; Valiashko, O.; Penco-Campillo, M.; Dufies, M.; Hagege, A.; Demange, L.; Martial, S.; Pagès, G.; Ronco, C.; Benhida, R. Synthesis and biological evaluation of 3-amino-1,2,4-triazole derivatives as potential anticancer compounds. *Bioorg. Chem.* **2020**, *104*, 104271. [[CrossRef](#)]
10. Li, S.M.; Tsai, S.E.; Chiang, C.Y.; Chung, C.Y.; Chuang, T.J.; Tseng, C.C.; Jiang, W.P.; Huang, G.J.; Lin, C.Y.; Yang, Y.C.; et al. New methyl 5-(halomethyl)-1-aryl-1H-1,2,4-triazole-3-carboxylates as selective COX-2 inhibitors and anti-inflammatory agents: Design, synthesis, biological evaluation, and docking study. *Bioorg. Chem.* **2020**, *104*, 104333. [[CrossRef](#)]
11. Khanage, S.G.; Raju, A.; Mohite, P.B.; Pandhare, R.B. Analgesic activity of some 1,2,4-triazole heterocycles clubbed with pyrazole, tetrazole, isoxazole and pyrimidine. *Adv. Pharm. Bull.* **2013**, *3*, 13–18.
12. Hichri, F.; Omri, A.; Hossan, A.S.M.; Ben Jannet, H. Alpha-glucosidase and amylase inhibitory effects of Eruca vesicaria subsp. longirostris essential oils: Synthesis of new 1,2,4-triazole-thiol derivatives and 1,3,4-thiadiazole with potential inhibitory activity. *Pharm. Biol.* **2019**, *57*, 564–570. [[CrossRef](#)] [[PubMed](#)]
13. Kaproń, B.; Łuszczki, J.J.; Siwek, A.; Karcz, T.; Nowak, G.; Zagaja, M.; Andres-Mach, M.; Stasiłowicz, A.; Cielecka-Piontek, J.; Kocki, J.; et al. Preclinical evaluation of 1,2,4-triazole-based compounds targeting voltage-gated sodium channels (VGSCs) as promising anticonvulsant drug candidates. *Bioorg. Chem.* **2020**, *94*, 103355. [[CrossRef](#)] [[PubMed](#)]
14. Aly, A.A.; Hassa, A.A.; Makhoul, M.M.; Bräse, S. Chemistry and biological activities of 1,2,4-Triazolethiones-antiviral and anti-infective drugs. *Molecules* **2020**, *25*, 3036. [[CrossRef](#)]
15. Navidpour, L.; Shabani, S.; Heidari, A.; Bashiri, M.; Ebrahim-Habibi, A.; Shahhosseini, S.; Shafaroodi, H.; Abbas Tabatabai, S.; Toolabi, M. 5-[Aryloxyphenyl] (or nitrophenyl)-4H-1,2,4-triazoles as novel flexible benzodiazepine analogues: Synthesis, receptor binding affinity and lipophilicity-dependent anti-seizure onset of action. *Bioorg. Chem.* **2021**, *106*, 104504. [[CrossRef](#)] [[PubMed](#)]
16. Al-Soud, Y.A.; Al-Dweri, M.N.; Al-Masoudi, N.A. Synthesis, antitumor and antiviral properties of some 1,2,4-triazole derivatives. *Farmaco* **2004**, *59*, 775–783. [[CrossRef](#)]
17. Xiao, H.; Li, P.; Guo, D.; Hu, J.; Chai, Y.; He, W. Synthesis and antibacterial activity evaluation of 2,6-bis(6-substituted-1,2,4-triazolo[3,4-b][1,3,4]thiadiazol-3-yl)pyridine derivatives. *Med. Chem. Res.* **2014**, *23*, 1941–1949. [[CrossRef](#)]
18. Shukla, P.K.; Soni, N.; Verma, A.; Jha, A.K. Synthesis, characterization and in vitro biological evaluation of a series of 1,2,4-triazoles derivatives & triazole based schiff bases. *Der Pharma Chem.* **2014**, *6*, 153–160.
19. Shi, Z.; Zhao, Z.; Huang, M.; Fu, X. Ultrasound-assisted, one-pot, three-component synthesis and antibacterial activities of novel indole derivatives containing 1,3,4-oxadiazole and 1,2,4-triazole moieties. *Comptes Rendus Chim.* **2015**, *18*, 1320–1327. [[CrossRef](#)]
20. Sadeghian, S.; Emami, L.; Mojaddami, A.; Khabnadideh, S.; Faghhi, Z.; Zomorodian, K.; Rashidi, M.; Rezaei, Z. 1,2,4-Triazole derivatives as novel and potent antifungal agents: Design, synthesis and biological evaluation. *J. Mol. Struct.* **2023**, *1271*, 134039. [[CrossRef](#)]
21. Dixit, D.; Verma, P.K.; Marwaha, R.K. A review on ‘triazoles’: Their chemistry, synthesis and pharmacological potentials. *J. Iran. Chem. Soc.* **2021**, *18*, 2535–2565. [[CrossRef](#)]
22. Bercean, V.N.; Creanga, A.A.; Badea, V.; Deleanu, C.; Csunderlik, C. (New) 5-Substituted-4H-4-amino-3-mercapto-1,2,4-triazoles with increased complexing capabilities. *Rev. Chim.* **2011**, *62*, 47–50.
23. Ledeti, I.V.; Bercean, V.N.; Tanase, I.M.; Creanga, A.A.; Badea, V.; Csunderlik, C. New Azomethine Derivatives of 3-substituted-4H-4-amino-5ethoxycarbonyl- methylsulfanyl-1,2,4-triazoles as Potential Anti-inflammatory Agents. *Rev. Chim.* **2010**, *61*, 937–939.

24. Bercean, V.N.; Turlea, M.L.; Badea, V.; Creanga, A.A.; Medeleanu, M. New 5-Substituted 2-Mercapto-1,3,4-Oxadiazoles, Intermediates in the Synthesis of 5-Substituted 4H-4-Amino-3-Mercapto-1,2,4-Triazoles. *Rev. Chim.* **2009**, *60*, 893–895.
25. Creanga, A.A.; Bercean, V.N.; Badea, V.; Patras, I.; Cocarta, A.; Tatu, C.; Csunderlik, C. Comparative Study for the Synthesis of Some 5-(2-, 3-, 4-pyridyl)Substituted-4H-4-Amino-3-Mercapto-1,2,4-Triazoles. *Rev. Chim.* **2010**, *61*, 1169–1172.
26. Ledeti, I.; Alexa, A.; Bercean, V.; Vlase, G.; Vlase, T.; Şuta, L.M.; Fuliş, A. Synthesis and degradation of Schiff bases containing heterocyclic pharmacophore. *Int. J. Mol. Sci.* **2015**, *16*, 1711–1727. [[CrossRef](#)] [[PubMed](#)]
27. Ratnakar, A.; Buchi Reddy, R.; Chandra Mouli, G.V.P.; Reddy, Y. Synthesis of a New Type of 5-Heteroaryl-3-Mercapto-4-Amino-1,2,4-Triazoles and their Derivatives. *Asian J. Chem.* **2010**, *4*, 197–200.
28. Burbuliene, M.M.; Jakubkiene, V.; Mekuskiene, G.; Vainilavicius, P. Synthesis of novel derivatives of 5-(4,6-dimethyl-2-pyrimidinylsulfanyl) methyl-1,2,4-triazole-3-thione. *Phosphorus Sulfur. Silicon Relat. Elem.* **2003**, *178*, 2431–2440. [[CrossRef](#)]
29. Mekuskiene, G.; Burbuliene, M.M.; Jakubkiene, V.; Udrenaite, E.; Gaidelis, P.; Vainilavicius, P. Reactions of 5-(4,6-Diphenyl-2-pyrimidinyl)-1,3,4-oxadiazole-2-thione with some C-electrophiles and N-nucleophiles. *Khimiya Geterotsiklicheskikh Soedin.* **2003**, *10*, 1548–1553.
30. Ghoneim, K.M.; Elmeligie, S.; Mohamed, M.S. New 2,4-disubstituted-3,5-dimethylpyrroles—Synthesis and reactions. *J. Indian. Chem. Soc.* **1995**, *72*, 829–832.
31. Elbarbary, A.A.; Anwar, M.; Ghattas, A.A.; Hashem, A.F. Reaction of 5-aryl-2-thiono-1,3,4-oxadiazoles with some amino-compounds and halo-compounds. *Rev. Roum. Chim.* **1981**, *26*, 449–456.
32. Shafiee, A.; Lalezari, I.; Mirrashed, M.; Nercesian, D. 1,2,3-Selenadiazolyl-1,3,4-oxadiazole, 1,2,3-Thiadiazolyl-1,3,4-oxadiazole and 5-(1,2,3-Thiadiazolyl)-s-triazolo [3,4-b]-1,3,4-thiadiazoles. *J. Heterocycl. Chem.* **1977**, *14*, 567–571. [[CrossRef](#)]
33. Giri, S.; Khan, M.H. Synthesis and Antifungal Activity of Some 3-[5'-Aryl-3'-Mercapto-1',2',4'-Triazol-4'-yl]-2-Aryl-4-Thiazolidinones. *Asian J. Chem.* **2010**, *4*, 812–817.
34. Hassan, E.; Al Ashmawi, M.I.; Abdel Fattah, B. Synthesis and antimicrobial testing of certain oxadiazoline and triazole derivatives. *Pharmazie* **1983**, *38*, 833–835.
35. Mortikov, V.Y.; Rodinovskaya, L.A.; Fedorov, A.E.; Shestopalov, A.M.; Belyakov, P.A. Synthesis of heterocyclic compounds from 4-formylpyrazoles. *Russ. Chem. Bull.* **2014**, *63*, 443–456. [[CrossRef](#)]
36. Liu, F.M.; Yu, J.X.; Lu, W.J.; Liu, G.; Liu, Y.T.; Chen, Y.Z. Synthesis of heterocyclic compounds from 2-phenyl-1,2,3-triazole-4-formylhydrazine. *Chin. J. Chem.* **1999**, *17*, 62–68. [[CrossRef](#)]
37. Raslan, M.A. Heterocyclic synthesis containing bridgehead nitrogen atom: Synthesis of 3-[(2H)-2-oxobenzo[b]pyran-3-yl]-s-triazolo[3,4-b]-1,3,4-thiadiazine and thiazole derivatives. *Heteroat. Chem.* **2003**, *14*, 114–120. [[CrossRef](#)]
38. Al-Soud, Y.A.; Al-Masoudi, N.A. A New Class of Dihaloquinolones Bearing N'-Aldehydoglycosylhydrazides, Mercapto-1,2,4-triazole, Oxadiazoline and α -Amino Ester Precursors: Synthesis and Antimicrobial Activity. *J. Braz. Chem. Soc.* **2003**, *14*, 790–796. [[CrossRef](#)]
39. Creanga, A.A. *Obținerea, Studiul și Proprietățile Mercaptanilor Heterociclici Din Clasa Azolilor, Compuși cu Potențial Efect Citotoxic*; Teze de doctorat; Editura Politehnica: Timișoara, Romania, 2010; ISBN 978-606-554-216-7.
40. Al-Amin, M.; Islam, M.R. Synthesis of some bis-triazole derivatives as probes for cytotoxicity study. *Bangladesh J. Pharmacol.* **2008**, *1*, 21–26. [[CrossRef](#)]
41. Riyadh, S.M.; Gomha, S.M. Two decades of the synthesis of mono- And bis-aminomercapto [1,2,4]triazoles. *RSC Adv.* **2020**, *10*, 24994–25012. [[CrossRef](#)]
42. Kudari, S.M.; Beede, S.M.; Munera, W. Synthesis and Biological Studies of Bis-Heterocycles. *Asian J. Chem.* **1997**, *9*, 20–26.
43. Shivarama Holla, B.; Gonsalves, R.; Shenoy, S. Studies on some N-bridged heterocycles derived from bis-[4-amino-5-mercapto-1,2,4-triazol-3-yl] alkanes. *Farm* **1998**, *53*, 574–578. [[CrossRef](#)] [[PubMed](#)]
44. Foroughifar, N.; Mobinikhaledi, A.; Ebrahimi, S. Synthesis of a Novel Class of Azacrown Macrocycles and Lariat Crown Ethers Containing Two 1,2,4-Triazole Rings as Subunits. *Synthesis* **2009**, *2009*, 2557–2560. [[CrossRef](#)]
45. Kharate, R.M.; Deohate, P.P.; Berad, B.N. Synthesis, structural study and antimicrobial screening of bridgehead nitrogen containing 1,8-bis-thiadiazino-triazolyl-octanes. *Der Pharma Chem.* **2012**, *4*, 2434–2437.
46. Smriti, D.; Singh, P. Synthesis and Fungicidal Activity of Bioactive 4,4'-bis [4''-(N-Benzylidinyllamine)-3''-mercapto-1'',2'',4''-triazole-5''-yl methoxy]dibenzyl. *Asian J. Chem.* **2017**, *29*, 19–21.
47. Curtius, T.; Jordan, H. Hydrazide und Azide organischer Säuren. *J. Prakt. Chem.* **2004**, *64*, 297–313. [[CrossRef](#)]
48. Oxalyl Dihydrazide on ChemBK. Available online: <https://www.chembk.com/en/chem/Oxalyl-dihydrazide> (accessed on 12 January 2024).
49. Malonic Dihydrazide on ChemBK. Available online: <https://www.chembk.com/en/chem/Malonic-dihydrazide> (accessed on 20 January 2024).
50. Succinic Dihydrazide on ChemBK. Available online: <https://www.chembk.com/en/chem/Succinic-dihydrazide;Butanedioyldihydrazide> (accessed on 20 January 2024).
51. Adipic Dihydrazide on Merck Millipore. Available online: https://www.merckmillipore.com/RO/ro/product/Adipic-dihydrazide,MDA_CHEM-841689?ReferrerURL=https://www.google.com/ (accessed on 20 January 2024).
52. Ahn, S.; Guo, F.; Kariuki, B.M.; Harris, K.D.M. Abundant polymorphism in a system with multiple hydrogen-bonding opportunities: Oxalyl dihydrazide. *J. Am. Chem. Soc.* **2006**, *128*, 8441–8452. [[CrossRef](#)] [[PubMed](#)]

53. Presti, D.; Pedone, A.; Menziani, M.C.; Civalleri, B.; Maschio, L. Oxalyl dihydrazide polymorphism: A periodic dispersion-corrected DFT and MP2 investigation. *CrystEngComm* **2014**, *16*, 102–109. [[CrossRef](#)]
54. Xu, P.-F.; Sun, X.-W.; Zhang, L.-M.; Zhang, Z.-Y. A Facile Synthesis of Bis(4-amino-5-mercapto-1,2,4-triazol-3-yl)alkanes and Bis(5-mercapto-4 H -1,2,4-triazol-3-yl)alkanes. *J. Chem. Res.* **1999**, *23*, 170–171. [[CrossRef](#)]

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