

Full Paper

Synthesis and Antimicrobial Activity of Some New 1,3,4-Thiadiazole and 1,2,4-Triazole Compounds Having a *D,L*-Methionine Moiety

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Abstract: New 1,3,4-thiadiazole, **5a-e**, and 1,2,4-triazole compounds **6a-c**, containing a *D,L*-methionine moiety were synthesized by intramolecular cyclization of 1,4-disubstituted thiosemicarbazides **4a-e** in acid and alkaline media, respectively. The potential antimicrobial effects of the synthesized compounds were investigated using the *Staphylococcus aureus* ATCC 25923, *Bacillus anthracis* ATCC 8705, *Bacillus cereus* ATCC 10987, *Sarcina lutea* ATCC 9341 and *Escherichia coli* ATCC 25922 strains. The newly synthesized compounds exhibited promising activities against *Bacillus anthracis* and *Bacillus cereus*.

Keywords: 1,3,4-Thiadiazole; 1,2,4-triazole; thiosemicarbazide; *D,L*-methionine; antimicrobial activity.

Introduction

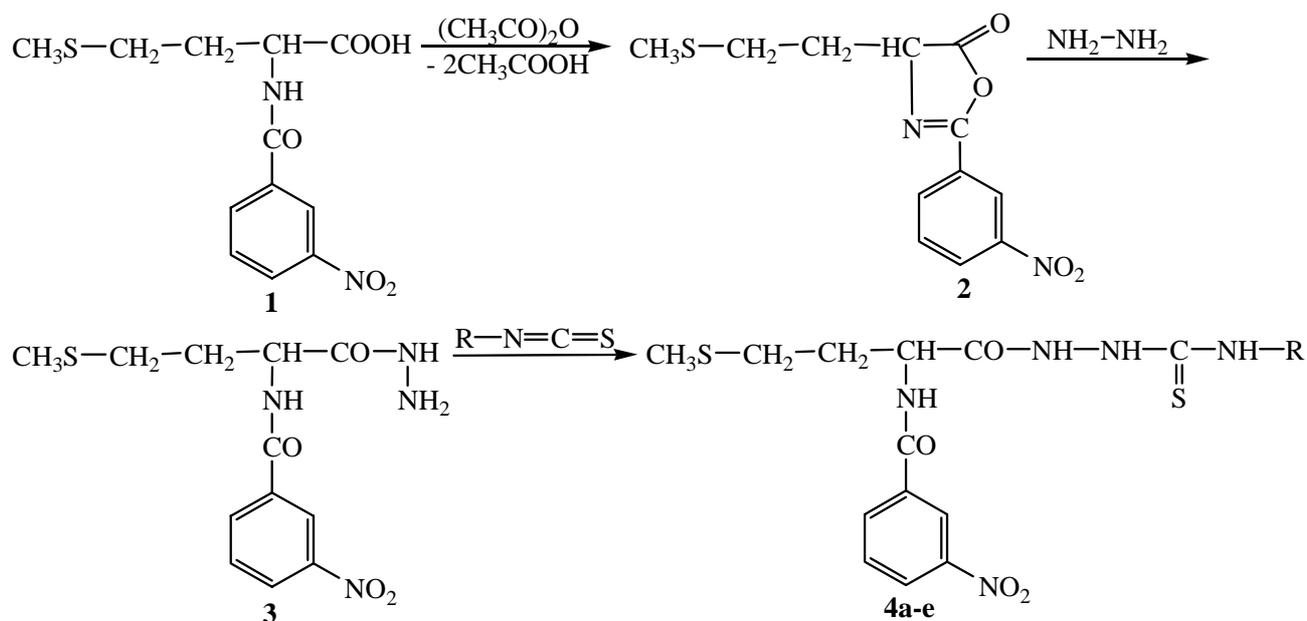
The therapeutic effects of compounds containing 1,3,4-thiadiazole and 1,2,4-triazole rings have been well studied for a number of pathological conditions including inflammation [1, 2], pain [3-5] or hypertension [6]. Moreover, synthesis of thiadiazoles and triazoles has attracted widespread attention due to their diverse applications as antibacterial [7], antimycobacterial [8, 9], antimycotic [10, 11], antifungal [12, 13] and antidepressant agents [14]. Meanwhile, *N*-acylated aminoacids are known for their hepatoprotective and antimicrobial effects [15, 16].

Taking these observations into account in the present study, some new 1,3,4-thiadiazoles and 1,2,4-triazoles having a *D,L*-methionine moiety have been synthesized and their structures were characterized by ¹H-NMR, IR spectroscopy and elemental analysis. The potential antimicrobial activity and degree of toxicity of the synthesized compounds were also investigated.

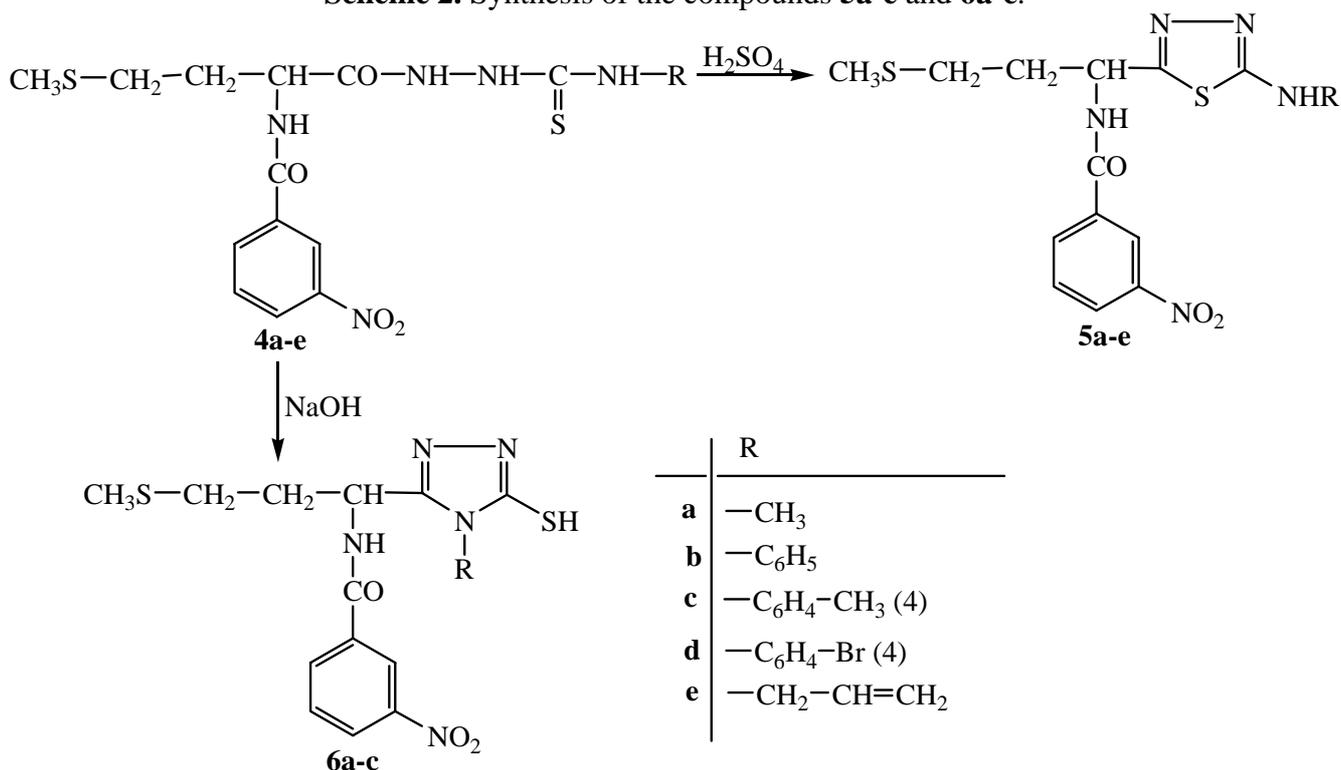
Results and Discussion

The synthesis of new 1,3,4-thiadiazole and 1,2,4-triazole compounds was performed in several steps. In the first step, 2-(3-nitrophenyl)-4-(2-methylthioethyl)- Δ^2 -5-oxazolinone (**2**) was obtained by cyclization of *N*-(3-nitrobenzoyl)-*D,L*-methionine (**1**) in the presence of acetic anhydride. This intermediate, through reaction with 98% hydrazine hydrate solution in a dioxane medium, gave the hydrazide of *N*-(3-nitrobenzoyl)-*D,L*-methionine (**3**), which, in the next step was reacted with different isothiocyanates whereupon new 1,4-disubstituted thiosemicarbazides **4a-e** were obtained (Scheme 1).

Scheme 1. Synthesis of the intermediates **4a-e**.



In the last step new 1,3,4-thiadiazole, **5a-e**, and 1,2,4-triazole compounds **6a-c** were obtained by intramolecular cyclization of the thiosemicarbazides **4a-e** in acid and alkaline media, respectively (Scheme 2). The structures of all the synthesized compounds were confirmed by IR, ¹H-NMR spectral measurements and elemental analysis.

Scheme 2. Synthesis of the compounds **5a-e** and **6a-c**.

The IR spectra of compounds **4a-e** showed intense absorption bands within the 3181–3367 cm⁻¹ range that were attributed to NH and NH₂ function vibrations. The absorption band of the —C=O function appears in the 1641–1644 cm⁻¹ region and the bands which appear at 1171–1173 cm⁻¹ were attributed to the —C=S function. In the ¹H-NMR spectra, the proton signals due to the NH group were recorded between 9.07–10.80 ppm and the aromatic protons signals appear at 7.19–8.74 ppm.

In the IR spectra of compounds **5a-e** the absorption band of the —NH-CO function appears at 2916–3302 cm⁻¹ and the absorption band of the C=N group was identified at 1431–1433 cm⁻¹. In the ¹H-NMR spectra the proton signal due to the —NH-CO function was observed as a singlet between 9.06–9.07 ppm.

The IR spectra of compounds **6a-c** showed an intense absorption band between 2589–2592 cm⁻¹ that was attributed to the —SH function and in the ¹H-NMR spectra, the proton signal due to the SH group appeared as a singlet at 12.60–12.80 ppm.

Moreover, the elemental analysis results were all in a good agreement with the structures proposed for compounds **2**, **3**, **4a-e**, **5a-e** and **6a-c**.

Biological activity

The potential antimicrobial activity of compounds **4a-e**, **5a-e** and **6a-c** towards five standard bacterial strains was investigated. From the data presented in Table 1 it may be seen that thiosemicarbazides **4a-e** exhibit relatively good activity against *Staphylococcus aureus* and *Escherichia coli*, but are only slightly active against *Bacillus anthracis*, *Bacillus cereus* and *Sarcina lutea* strains. Upon cyclization of thiosemicarbazides to the corresponding thiadiazoles **5a-e** and triazoles **6a-c** the activity towards *Staphylococcus aureus* and *Escherichia coli* decreases, while the

activity against *Bacillus antracis* and *Bacillus cereus* increases. Concerning the activity towards the *Sarcina lutea* strain, it was noted that the activity of the triazoles is comparable to that of the thiosemicarbazideas, but the thiadiazoles are inactive. The most active compounds against *Bacillus antracis* and *Bacillus cereus* are **5c** (a thiadiazole compound) and **6c** (a triazole compound), both with a 4-methylphenyl moiety on the heterocyclic ring. This could be explained by electropositive effect of methyl group attached to the phenyl moiety because of the known favorable influence of electron donating groups on the potency of the heterocyclic nuclei. Further investigations are in progress.

Table 1. Antimicrobial activity of compounds **4a-e**, **5a-e** and **6a-c**.

Comp. no.	Minimum Inhibitory Concentration (MIC) ($\mu\text{g/mL}$)				
	<i>Sa</i>	<i>Ba</i>	<i>Bc</i>	<i>Sl</i>	<i>Ec</i>
4a	171	618	714	638	193
4b	113	620	774	782	185
4c	105	587	898	761	201
4d	126	613	883	791	198
4e	138	636	716	829	228
5a	732	143	186	1264	734
5b	489	137	194	1137	836
5c	642	119	128	1142	869
5d	529	131	147	1153	765
5e	725	130	173	1215	892
6a	852	142	168	615	759
6b	748	154	205	549	629
6c	456	122	131	521	858

(*Sa*): *Staphylococcus aureus* ATCC 25923; (*Ba*): *Bacillus antracis* ATCC 8705; (*Bc*): *Bacillus cereus* ATCC 10987; (*Sl*): *Sarcina lutea* ATCC 9341; (*Ec*) *Escherichia coli* ATCC 25922.

The synthesized compounds were also investigated for their toxicity (Table 2) and it was observed that all tested compounds had a low toxicity.

Table 2. The DL50 values of the tested compounds.

Comp.	DL ₅₀ mg/Kg body	Comp.	DL ₅₀ mg/Kg body
4a	1465	5c	3100
4b	1275	5d	2025
4c	1625	5e	2816
4d	1315	6a	4620
4e	1260	6b	5010
5a	1825	6c	4920
5b	2110		

Conclusions

New 1,3,4-thiadiazole, **5a-e**, and 1,2,4-triazole compounds **6a-c** possessing a *D,L*-methionine moiety were synthesized by intramolecular cyclization of 1,4-disubstituted thiosemicarbazides in acid and alkaline media, respectively. The potential antimicrobial effects of the synthesized compounds were investigated using the *Staphylococcus aureus* ATCC 25923, *Bacillus anthracis* ATCC 8705, *Bacillus cereus* ATCC 10987, *Sarcina lutea* ATCC 9341 and *Escherichia coli* ATCC 25922 strains. The most active compounds were **5c** and **6c** containing a 4-methylphenyl substituent on the heterocyclic ring, which exhibited promising activities against *Bacillus anthracis* and *Bacillus cereus*.

Experimental Section

General

All melting points were determined on a Melt-Temp R apparatus equipped with a digital thermometer and are uncorrected. The combustion analysis was performed on an Elemental Exeter Analytical CE 440 Apparatus. The IR spectra were measured as potassium bromide pellets on a Digilab Scimitar Series spectrophotometer; the wave numbers are given in cm^{-1} . The $^1\text{H-NMR}$ spectra were recorded in DMSO-d_6 solutions on Bruker ARX-300 spectrometer (^1H : 300 MHz) at ambient temperature. Chemical shifts were recorded as δ values in parts per millions (ppm) and were indirectly referenced to tetramethylsilane via the residual solvent signal (2.49 for ^1H). All chemical reagents were obtained from the Aldrich Chemical Company.

Synthesis of 2-(3-nitrophenyl)-4-(2-methylthioethyl)- Δ^2 -5-oxazolinone (**2**).

N-(3-Nitrobenzoyl)-*D,L*-methionine (5.6 g, 0.018 mol) was dissolved in acetic anhydride (15 mL) and heated at 65-70 °C for half an hour. After cooling the solution was added under stirring to a mixture of petroleum ether (50 mL) and dried ethyl ether (10 mL). The reaction mixture was stirred for 30 minutes, the ethereal layer was removed and the oily product obtained was washed with petroleum ether. The crude product was dissolved in dioxane and crystallized from anhydrous ethyl ether – anhydrous petroleum ether (1:1). The solid compound obtained was dried under vacuum at 40-45 °C. Yield 76.20 %; m.p. 114-115 °C; Anal. Calc. for $\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}_4\text{S}$ (280.05): 51.42% C, 4.28% H, 10.00% N, 11.42% S; found 51.44% C, 4.01% H, 9.81% N, 11.26% S; IR (ν , cm^{-1}): 725 (CH_3S), 819 (aromatic CH), 1350 (symmetric vibrations of NO_2), 1433 ($-\text{C}=\text{N}$), 1529 (asymmetric vibrations of NO_2), 1707 (CO). $^1\text{H-NMR}$ δ : 2.09 (s, 3H, CH_3), 2.52 (m, 2H, CH_2), 2.65 (m, 2H, CH_2), 4.50 (t, 1H, CH), 7.80 (s, 1H, ArH), 8.30 (d, 1H, ArH), 8.40 (d, 1H, ArH), 8.70 (s, 1H, ArH).

N-(3-nitrobenzoyl)-*D,L*-methionyl-hydrazide (**3**).

The 2-(3-nitrophenyl)-4-(2-methylthioethyl)- Δ^2 -5-oxazolinone (**2**, 1 g, 0.035 mol) was dissolved in dioxane (10 mL) and aqueous hydrazine hydrate solution (98%, 0.172 mL, 0.0035 mol) was added. The reaction mixture was heated at 65-70 °C for one hour. After cooling the solvent was removed and

the obtained oily product was dissolved in absolute ethanol and precipitated with water. The solid compound was dried under vacuum at 40-45 °C. The crude product was crystallized from ethanol-water. Yield 54.78 %; m.p. 102-104 °C; Anal. Calc. for C₁₂H₁₆N₄O₄S (327.11): 46.15% C, 5.12% H, 17.94% N, 11.42% S; found 46.02% C, 4.98% H, 17.89% N, 11.26% S; IR (v, cm⁻¹): 716 (CH₃S); 818 (aromatic CH); 1352 (symmetric vibrations of NO₂); 1527 (asymmetric vibrations of NO₂); 1638 (CO); 2916, 3103, 3368 (NH); ¹H-NMR δ: 2.10 (s, 3H, CH₃); 2.50 (m, 4H, CH₂); 4.50 (t, 1H, CH); 7.80 (t, 1H, Ar); 8.34 (d, 1H, Ar); 8.42 (d, 1H, Ar); 8.74 (s, 1H, Ar); 9.05 (d, 1H, NH); 10.80 (s, 1H, NH); 11.20 (s, 2H, NH₂).

General procedure for the synthesis of 1,4-disubstituted thiosemicarbazides 4a-e.

N-(3-nitrobenzoyl)-*D,L*-methionylhydrazide (**3**, 0.78 g, 0.0025 mol) was dissolved in methanol (10 mL) and a solution of the appropriate isothiocyanate (0.0025 mol) in methanol (10 mL) was added. The reaction mixture was heated at 70-80 °C for two hours. After cooling the solvent was evaporated under reduced pressure and the solid was dried under vacuum at room temperature. The crude product was purified by crystallization from ethanol.

1-[*N*-(3-nitrobenzoyl)-*D,L*-methionyl]-4-methyl-thiosemicarbazide (**4a**). Yield 58.33 %; m.p. 121-122 °C; Anal. Calc. for C₁₄H₁₉N₅O₄S₂ (385.09): 43.63% C, 4.93% H, 18.18% N, 16.62% S; found 43.59% C, 4.75% H, 18.03% N, 16.41% S; IR (v, cm⁻¹): 720 (CH₃S); 819 (aromatic CH); 1173 (C=S); 1351 (symmetric vibrations of NO₂); 1528 (asymmetric vibrations of NO₂); 1643 (CO); 3302-3367 (NH-CO); ¹H-NMR δ: 2.11 (s, 3H, CH₃); 2.51 (d, 3H, CH₃); 2.54 (m, 4H, CH₂); 4.50 (t, 1H, CH); 7.80 (t, 1H, Ar); 8.30 (d, 1H, Ar); 8.40 (d, 1H, Ar); 8.70 (s, 1H, Ar); 9.07 (s, 1H, NH); 9.80 (m, 1H, NH); 10.10 (s, 1H, NH).

1-[*N*-(3-nitrobenzoyl)-*D,L*-methionyl]-4-phenyl-thiosemicarbazide (**4b**). Yield 68 %; m.p. 110-111 °C; Anal. Calc. for C₁₉H₂₁N₅O₄S₂ (447.10): 51.00% C, 4.69% H, 15.65% N, 14.31% S; found 49.72% C, 4.48% H, 15.37% N, 14.25% S; IR (v, cm⁻¹): 721 (CH₃S); 823 (aromatic CH); 1173 (C=S); 1349 (symmetric vibrations of NO₂); 1532 (asymmetric vibrations of NO₂); 1643 (CO); 3181- 3301 (NH-CO); ¹H-NMR δ: 2.09 (s, 3H, CH₃); 2.50 (m, 4H, CH₂); 4.50 (t, 1H, CH); 7.19 (t, 1H, Ar); 7.29 (t, 2H, Ar); 7.38 (d, 2H, Ar); 7.81 (t, 1H, Ar); 8.41 (d, 1H, Ar); 8.47 (d, 1H, Ar); 8.70 (s, 1H, Ar); 9.07 (s, 1H, NH); 9.81 (s, 1H, NH); 10.10 (s, 1H, NH).

1-[*N*-(3-nitrobenzoyl)-*D,L*-methionyl]-4-(4-methylphenyl)-thiosemicarbazide (**4c**). Yield 78.35 %; m.p. 112-113 °C; Anal. Calc. for C₂₀H₂₃N₅O₄S₂ (461.12): 52.06% C, 4.98% H, 15.18% N, 13.88% S; found 51.92% C, 4.75% H, 15.01% N, 13.58% S; IR (v, cm⁻¹): 721 (CH₃S); 819 (aromatic CH); 1173 (C=S); 1350 (symmetric vibrations of NO₂); 1528 (asymmetric vibrations of NO₂); 1644 (CO); 3301-3366 (NH-CO); ¹H-NMR δ: 2.11 (s, 3H, CH₃); 2.43 (s, 3H, CH₃); 2.50 (m, 4H, CH₂); 4.50 (t, 1H, CH); 7.80 (t, 1H, Ar); 8.12 (d, 2H, Ar); 8.32 (d, 2H, Ar); 8.39 (d, 1H, Ar); 8.41 (d, 1H, Ar); 8.74 (s, 1H, Ar); 9.07 (d, 1H, NH); 9.80 (s, 1H, NH); 10.10 (s, 1H, NH).

1-[N-(3-nitrobenzoyl)-D,L-methionyl]-4-(4-bromophenyl)-thiosemicarbazide (4d). Yield 73.05 %; m.p. 95-96 °C; Anal. Calc. for C₁₉H₂₀N₅O₄S₂Br (525.01): 43.34% C, 3.80% H, 13.30% N, 12.16% S; found 43.18% C, 3.72% H, 13.05% N, 12.11% S; IR (v, cm⁻¹): 718 (CH₃S); 819 (aromatic CH); 1171 (C=S); 1350 (symmetric vibrations of NO₂); 1528 (asymmetric vibrations of NO₂); 1641 (CO); 3101-3300 (NH-CO); ¹H-NMR δ: 2.11 (s, 3H, CH₃); 2.50 (m, 4H, CH₂); 4.50 (t, 1H, CH); 7.80 (t, 1H, Ar); 8.17 (d, 2H, Ar); 8.30 (d, 2H, Ar); 8.39 (d, 1H, Ar); 8.45 (d, 1H, Ar); 8.70 (s, 1H, Ar); 9.07 (d, 1H, NH); 9.81 (s, 1H, NH); 10.10 (s, 1H, NH).

1-[N-(3-nitrobenzoyl)-D,L-methionyl]-4-allyl-thiosemicarbazide (4e). Yield 76.27 %; m.p. 117-118 °C; Anal. Calc. for C₁₆H₂₁N₅O₄S₂ (411.10): 46.71% C, 5.10% H, 17.03% N, 15.57% S; found 46.48% C, 5.02% H, 16.98% N, 15.23% S; IR (v, cm⁻¹): 768 (CH₃S); 823 (aromatic CH); 1172 (C=S); 1348 (symmetric vibrations of NO₂); 1529 (asymmetric vibrations of NO₂); 1644 (CO); 3082-3302 (NH-CO); ¹H-NMR δ: 1.80 (d, 2H, CH₂); 2.11 (s, 3H, CH₃); 2.50 (m, 4H, CH₂); 4.50 (t, 1H, CH); 5.90 (t, 2H, CH₂); 6.32 (m, 1H, CH); 7.80 (t, 1H, Ar); 8.34 (d, 1H, Ar); 8.47 (d, 1H, Ar); 8.74 (s, 1H, Ar); 9.07 (d, 1H, NH); 9.82 (t, 1H, NH); 10.10, 10.30 (s, 1H, NH).

General procedure for the synthesis of 1,3,4-thiadiazole compounds 5a-e.

To corresponding thiosemicarbazide **4a-e** (0.006 mol) concentrated H₂SO₄ (1 mL) was added under stirring. The reaction mixture was stirred at room temperature for one hour and then added dropwise to cold water. The obtained solid was dried under vacuum at 45 °C. The crude product was purified by crystallization from ethanol.

2-[1-(3-nitrobenzoylamino)-3-(methylthio)]-propyl-5-(methylamino)-1,3,4-thiadiazole (5a). Yield 69.76 %; m.p. 116-117 °C; Anal. Calc. for C₁₄H₁₇N₅O₃S₂ (367.08): 45.77% C, 4.63% H, 19.07% N, 17.43% S; found 45.55% C, 4.58% H, 18.92% N, 17.21% S; IR (v, cm⁻¹): 725 (CH₃S); 821 (aromatic CH); 1350 (symmetric vibrations of NO₂); 1433 (C=N); 1527 (asymmetric vibrations of NO₂); 1645 (CO); 3302 (NH-CO); ¹H-NMR δ: 2.06 (s, 3H, CH₃); 2.50 (m, 4H, CH₂); 2.80 (d, 3H, CH₃); 4.50 (t, 1H, CH); 7.06 (m, 1H, NH); 7.80 (t, 1H, Ar); 8.30 (d, 1H, Ar); 8.40 (d, 1H, Ar); 8.70 (s, 1H, Ar); 9.07 (d, 1H, NH).

2-[1-(3-nitrobenzoylamino)-3-(methylthio)]-propyl-5-(phenylamino)-1,3,4-thiadiazole (5b). Yield 68.72 %; m.p. 105-106 °C; Anal. Calc. for C₁₉H₁₉N₅O₃S₂ (429.09): 53.11% C, 4.42% H, 16.31% N, 14.91% S; found 52.98% C, 4.38% H, 16.22% N, 14.81% S; IR (v, cm⁻¹): 725 (CH₃S); 821 (aromatic CH); 1350 (symmetric vibrations of NO₂); 1433 (C=N); 1527 (asymmetric vibrations of NO₂); 1645 (CO); 2916-3302 (NH-CO); ¹H-NMR δ: 2.09 (s, 3H, CH₃); 2.50 (m, 4H, CH₂); 4.50 (t, 1H, CH); 7.10 (t, 1H, Ar); 7.20 (t, 2H, Ar); 7.30 (d, 2H, Ar); 7.80 (t, 1H, Ar); 8.41 (d, 1H, Ar); 8.47 (d, 1H, Ar); 8.74 (s, 1H, Ar); 9.06 (d, 1H, NH); 10.43 (s, 1H, NH).

2-[1-(3-nitrobenzoylamino)-3-(methylthio)]-propyl-5-(4-methylphenylamino)-1,3,4-thiadiazole (5c). Yield 62.43 %; m.p. 98-99 °C; Anal. Calc. for C₂₀H₂₁N₅O₃S₂ (443.11): 54.17% C, 4.74% H, 15.80% N, 14.44% S; found 54.12% C, 4.38% H, 15.72% N, 14.28% S; IR (v, cm⁻¹): 725 (CH₃S); 819

(aromatic CH); 1350 (symmetric vibrations of NO₂); 1433 (C=N); 1527 (asymmetric vibrations of NO₂); 1645 (CO); 3084-3302 (NH-CO); ¹H-NMR δ: 2.09 (s, 3H, CH₃); 2.50 (m, 4H, CH₂); 4.50 (t, 1H, CH); 7.10 (s, 3H, CH₃); 7.78 (t, 1H, Ar); 7.82 (d, 2H, Ar); 7.94 (d, 2H, Ar); 8.30 (d, 1H, Ar); 8.40 (d, 1H, Ar); 8.00 (s, 1H, Ar); 9.07 (d, 1H, NH); 10.43 (s, 1H, NH).

2-[1-(3-nitrobenzoylamino)-3-(methylthio)]-propyl-5-(4-bromophenylamino)-1,3,4-thiadiazole (5d). Yield 61.18 %; m.p. 106-107 °C; Anal. Calc. for C₁₉H₁₈N₅O₃S₂Br (507.00): 44.88% C, 3.54% H, 13.77% N, 12.59% S; found 44.81% C, 3.25% H, 13.51% N, 12.42% S; IR (ν, cm⁻¹): 769 (CH₃S); 825 (aromatic CH); 1349 (symmetric vibrations of NO₂); 1432 (C=N); 1529 (asymmetric vibrations of NO₂); 1644 (CO); 3082-3302 (NH-CO); ¹H-NMR δ: 2.11 (s, 3H, CH₃); 2.50 (m, 4H, CH₂); 4.50 (t, 1H, CH); 7.90 (t, 1H, Ar); 8.05 (d, 2H, Ar); 8.08 (d, 2H, Ar); 8.41 (d, 1H, Ar); 8.47 (d, 1H, Ar); 8.74 (s, 1H, Ar); 9.07 (d, 1H, NH); 10.43 (s, 1H, NH).

2-[1-(3-nitrobenzoylamino)-3-(methylthio)]-propyl-5-(allylamino)-1,3,4-thiadiazole (5e). Yield 71.03 %; m.p. 112-113 °C; Anal. Calc. for C₁₆H₁₉N₅O₃S₂ (393.09): 48.85% C, 4.83% H, 17.81% N, 16.28% S; found 48.69% C, 4.78% H, 17.62% N, 16.01% S; IR (ν, cm⁻¹): 723 (CH₃S); 819 (aromatic CH); 1350 symmetric vibrations of (NO₂); 1431 (C=N); 1527 (asymmetric vibrations of NO₂); 1645 (CO); 3082-3300 (NH-CO); ¹H-NMR δ: 2.06 (s, 3H, CH₃); 2.50 (m, 4H, CH₂); 3.70 (d, 2H, CH₂); 4.50 (t, 1H, CH); 5.90 (t, 2H, CH₂); 6.30 (m, 1H, CH); 7.80 (t, 1H, Ar); 8.30 (d, 1H, Ar); 8.40 (d, 1H, Ar); 8.70 (s, 1H, Ar); 9.07 (d, 1H, NH); 10.42 (t, 1H, NH).

General procedure for the synthesis of 1,2,4-triazole compounds 6a-c.

To corresponding thiosemicarbazide **4a-e** (0.0014 mol) a solution of NaOH 2N (10 mL) was added. The reaction mixture was heated at 80 °C for one hour, diluted with water (1:1) and then a solution of HCl 1N was added till pH 4.5. The obtained solids were dried at 45 °C and then recrystallized from ethanol.

3-mercapto-4-methyl-5-[1-(3-nitrobenzoylamino)-3-methylthio]-propyl-1,2,4-triazole (6a). Yield 62.43 %; m.p. 280-281 °C; Anal. Calc. for C₁₄H₁₇N₅O₃S₂ (367.08): 45.77% C, 4.63% H, 19.07% N, 17.43% S; found 45.62% C, 4.48% H, 18.93% N, 17.21% S; IR (ν, cm⁻¹): 686 (CH₃S); 866 (aromatic CH); 1350 (symmetric vibrations of NO₂); 1436 (C=N); 1580 (asymmetric vibrations of NO₂); 1648 (CO); 2592 (SH); 2974-3271 (NH-CO); ¹H-NMR δ: 2.11 (s, 3H, CH₃); 2.50 (m, 4H, CH₂); 2.80 (s, 3H, CH₃); 4.50 (t, 1H, CH); 7.80 (t, 1H, Ar); 8.41 (d, 1H, Ar); 8.47 (d, 1H, Ar); 8.74 (s, 1H, Ar); 9.05 (d, 1H, NH); 12.60 (s, 1H, SH).

3-mercapto-4-phenyl-5-[1-(3-nitrobenzoylamino)-3-methylthio]-propyl-1,2,4-triazole (6b). Yield 60.31 %; m.p. 233-234 °C; Anal. Calc. for C₁₉H₁₉N₅O₃S₂ (429.09): 53.14% C, 4.42% H, 16.31% N, 14.91% S; found 53.02% C, 4.16% H, 16.28% N, 14.85% S; IR (ν, cm⁻¹): 684 (CH₃S); 866 (aromatic CH); 1348 (symmetric vibrations of NO₂); 1440 (C=N); 1550 (asymmetric vibrations of NO₂); 1691 (CO); 2589 (SH); 2981-3462 (NH-CO); ¹H-NMR δ: 2.08 (s, 3H, CH₃); 2.50 (m, 4H, CH₂); 4.50 (t, 1H,

CH); 7.18 (t, 1H, Ar); 7.27 (t, 2H, Ar); 7.38 (d, 2H, Ar); 7.80 (t, 1H, Ar); 8.30 (d, 1H, Ar); 8.40 (d, 1H, Ar); 8.70 (s, 1H, Ar); 9.07 (d, 1H, NH); 12.80 (s, 1H, SH).

3-mercapto-4-(4-methylphenyl)-5-[1-(3-nitrobenzoylamino)-3-methylthio]-propyl-1,2,4-triazole (6c). Yield 68.38 %; m.p. 285-286 °C; Anal. Calc. for C₂₀H₁₉N₅O₃S₂ (443.11): 54.17% C, 4.74% H, 15.80% N, 14.44% S; found 54.03% C, 4.58% H, 15.52% N, 14.39% S; IR (v, cm⁻¹): 686 (CH₃S); 866 (aromatic CH); 1350 (symmetric vibrations of NO₂); 1450 (C=N); 1580 (asymmetric vibrations of NO₂); 1698 (CO); 259 (SH); 2976-3486 (NH-CO); ¹H-NMR δ: 2.06 (s, 3H, CH₃); 2.50 (m, 4H, CH₂); 4.50 (t, 1H, CH); 7.10 (s, 3H, CH₃); 7.80 (t, 1H, Ar); 7.90 (d, 2H, Ar); 8.08 (d, 2H, Ar); 8.41 (d, 1H, Ar); 8.47 (d, 1H, Ar); 8.74 (s, 1H, Ar); 9.07 (d, 1H, NH); 12.70 (s, 1H, SH).

Antimicrobial activity assessment

The test microorganisms used to evaluate the potential antimicrobial activity of the new synthesized compounds were: *Staphylococcus aureus* ATCC 25923, *Bacillus antracis* ATCC 8705, *Bacillus cereus* ATCC 10987, *Sarcina lutea* ATCC 9341 and *Escherichia coli* ATCC 25922. All the new compounds were weighed and dissolved in dimethylsulphoxide (DMSO) to prepare an extract stock solution of 100 mg/mL. The antimicrobial effects of the substances were quantitatively tested in the respective broth media by using double dilution and the Minimal Inhibitory Concentration (MIC) values (µg/mL) were determined [17]. The antibacterial assays were performed in Mueller-Hinton broth (MH) at pH 7.3. The MIC was defined as the lowest concentration that showed no growth. Dimethylsulfoxide (DMSO) with dilution of 1:10 was used as solvent control.

Toxicity study

The acute toxicity was estimated by intraperitoneal administration of the compounds as a suspension in Tween 80 to groups of fourteen mice, each weighting 20-25 g, according to the classical laboratory methodology [18]. The animals were observed and the death rate ascertained after 7 days.

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Sample Availability: Samples of the compounds **5a-e** and **6a-c** are available from authors.

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