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New Derivatives of Thiosemicarbazide and 1,2,4-Triazoline-5-thione with Potential Antimicrobial Activity

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*In the reaction of the hydrazide of (4-nitroimidazol-1-yl)acetic acid **1** with isothiocyanates, the respective thiosemicarbazide derivatives **2–6** were obtained. Further cyclization with 2% NaOH led to the formation of 3-[(4-nitroimidazol-1-yl)-methyl]-4-substituted-1,2,4-triazoline-5-thiones **7–11**. The structures of all new products were confirmed by analytical and spectroscopic methods. Six compounds **2–5**, **8**, and **10** were screened for their *in vitro* activity against some species of aerobic bacteria and fungi. Compound **3** appears to be a promising precursor of agents with antibacterial activity against *Micrococcus luteus*.*

Keywords Antimicrobial activity; isothiocyanates; thiosemicarbazides; 1,2,4-triazoles

INTRODUCTION

1,2,4-Triazoles have been reported to exhibit antimicrobial, fungicidal, anti-inflammatory, antiparasitic, insecticidal, herbicidal, antiviral, antitumor, anticonvulsant, antidepressant, and hypotensive effects and plant growth regulatory activities.^{1–8} Alternatively, nitroimidazoles such as metronidazole, ornidazole, secnidazole, and tinidazole are widely used in the treatment of diseases caused by protozoa and anaerobic bacteria.^{9–12} Furthermore, some 5-nitroimidazoles have been shown to be active against *Helicobacter pylori*.^{13–14} It has also been shown that the presence of the 4-nitroimidazole substituent in the triazole, oxadiazole, and thiadiazole ring was the most important reason for antibacterial activity of the tested compounds.¹⁵ This article is a continuation of investigations of compounds with

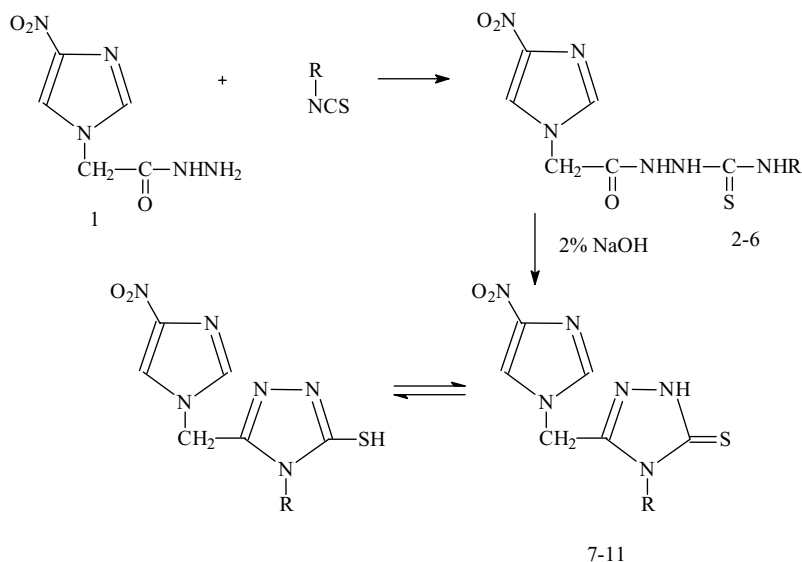
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promising antimicrobial activities.^{16–17} In this work, we present new analogous of 4-nitroimidazole, 3-[(4-nitroimidazol-1-yl)methyl]-4-substituted-1,2,4-triazoline-5-thione.¹⁵ It was very interesting to investigate if the exchange of substituent in the position 4 of 3-[(4-nitroimidazol-1-yl)methyl]-1,2,4-triazoline-5-thione will influence antimicrobial activity.

RESULTS AND DISCUSSION

We have used (4-nitroimidazol-1-yl)acetic acid hydrazide **1** as the starting material for the synthesis of new derivatives of 1,2,4-triazole-5-thione **7–11**. It was obtained in the reaction of ethyl (4-nitroimidazol-1-yl)acetate with 80% hydrazine hydrate. New thiosemicarbazide derivatives **2–6** were obtained by the reaction of **1** with isothiocyanates. The conditions of the reaction were established experimentally. Thiosemicarbazides **2–6** were subjected to cyclization in 2% solution of sodium hydroxide yielding the corresponding 3-[(4-nitroimidazol-1-yl)methyl]-4-substituted-1,2,4-triazoline-5-thiones **7–11**. The reactions were performed according to Scheme 1.



SCHEME 1

The structures of the obtained products were confirmed by elemental analysis as well as by IR and ^1H NMR spectra. Substituents

TABLE I Substituents and Yields of Thiosemicarbazide Derivatives

	Intermediate R	Yield [%]
2	C ₆ H ₅	70
3	4-CH ₃ C ₆ H ₄	75
4	4-CH ₃ OC ₆ H ₄	78
5	4-BrC ₆ H ₄	75
6	4-IC ₆ H ₄	70

and corresponding yields of intermediates **2–6** and products **7–11** are collected in Tables I and II, respectively. As indicated in Scheme 1, products of the cyclization can exist in two major tautomeric forms: thiole (the structure on the left) and thione. In ¹H NMR spectra of compounds **7–11** proton signals for –NH group in the δ (ppm) 13.10–14.02 range were observed. The above observations suggest that the compounds exist mainly in the thione form.

The in vitro antimicrobial activities of the newly synthesized compounds **2–5**, **8**, and **10** at concentrations ranging from 500 to 31.25 mg L^{–1} were screened using the agar dilution method against four Gram-negative and five Gram-positive reference species of bacteria and five species of fungi. According to our preliminary results, among the tested compounds, a few of them possessed some antibacterial (compounds **2**, **3**, **10**) or antifungal (compound **3**) activity.

Using agar and broth dilution methods, it was found that compounds **3** and **10** inhibited the growth of Gram-positive *Micrococcus luteus* ATCC 10240 with MIC = 250 mg L^{–1} and MIC = 500 mg L^{–1}, respectively (Table III). Compound **2** was effective against Gram-negative

TABLE II Substituents and Yields of 1,2,4-Triazoline-5-thione Derivatives

	Product R	Yield [%]
7	C ₆ H ₅	85
8	4-CH ₃ C ₆ H ₄	79
9	4-CH ₃ OC ₆ H ₄	80
10	4-BrC ₆ H ₄	75
11	4-IC ₆ H ₄	70

TABLE III The Percentage of Growth Inhibitory Effect of the Tested Compounds 2, 3, and 10 at Selected Concentrations

Compound	mgL ⁻¹	Microorganisms									
		Se	Sa ¹	Sa ²	Bs	Bc	MI	Ec	Kp	Pm	Pa
2	500	0	0	0	0	0	0	100	68	36	0
	250	0	0	0	0	0	0	65	62	22	0
	125	0	0	0	0	0	0	58	60	12	0
	62.5	0	0	0	0	0	0	54	56	11	0
	31.25	0	0	0	0	0	0	49	53	0	0
3	500	0	0	0	0	0	100	0	0	0	0
	250	0	0	0	0	0	100	0	0	0	0
	125	0	0	0	0	0	67	0	0	0	0
	62.5	0	0	0	0	0	40	0	0	0	0
	31.25	0	0	0	0	0	14	0	0	0	0
10	500	0	0	0	0	0	100	0	0	0	0
	250	0	0	0	0	0	73	0	0	0	0
	125	0	0	0	0	0	37	0	0	0	0
	62.5	0	0	0	0	0	30	0	0	0	0
	31.25	0	0	0	0	0	11	0	0	0	0

Abbreviations: Se – *Staphylococcus epidermidis* ATCC 12228 (n = 0.488), Sa¹ – *Staphylococcus aureus* ATCC 25923 (n = 0.474), Sa² – *Staphylococcus aureus* ATCC (n = 0.489), Bs – *Bacillus subtilis* ATCC 6633 (n = 0.222), Bc – *Bacillus cereus* ATCC 10876 (n = 0.289), MI – *Micrococcus luteus* ATCC 10240 (n = 0.306), Ec – *Escherichia coli* ATCC 25922 (n = 0.570), *Klebsiella pneumoniae* ATCC 13883 (n = 0.378), Pm – *Proteus mirabilis* ATCC 12453 (n = 0.469), Pa – *Pseudomonas aeruginosa* ATCC 9027 (n = 0.613), n = control growth of tested bacteria in broth without compound (OD₆₀₀).

bacteria with MIC = 500 mg L⁻¹ for *E. coli* ATCC 25922. Furthermore, this compound caused partial reduction of the growth (about 36–65% inhibition) of *Klebsiella pneumoniae* ATCC 13883 and *Proteus mirabilis* ATCC 12453 at lower concentrations ranging from 7.81 to 250 mg L⁻¹ without any activity against remaining Gram-negative (*Pseudomonas aeruginosa* ATCC 9027) or Gram-positive (*Staphylococcus* spp. and *Bacillus* spp.) bacteria (Table III).

Compound 3 showed inhibitory effect against *Trichophyton menthagrophytes* ATCC 9533 with MIC = 250 mg L⁻¹; in addition, this compound caused partial (about 5–70%) inhibition of the growth of *Candida* spp. and *Aspergillus niger* ATCC 16404 at 31.25–500 mg L⁻¹ concentrations (Figure 1).

The antimicrobial result demonstrated that it is worth obtaining research in this group of compounds for more activity against Gram-positive and fungi.

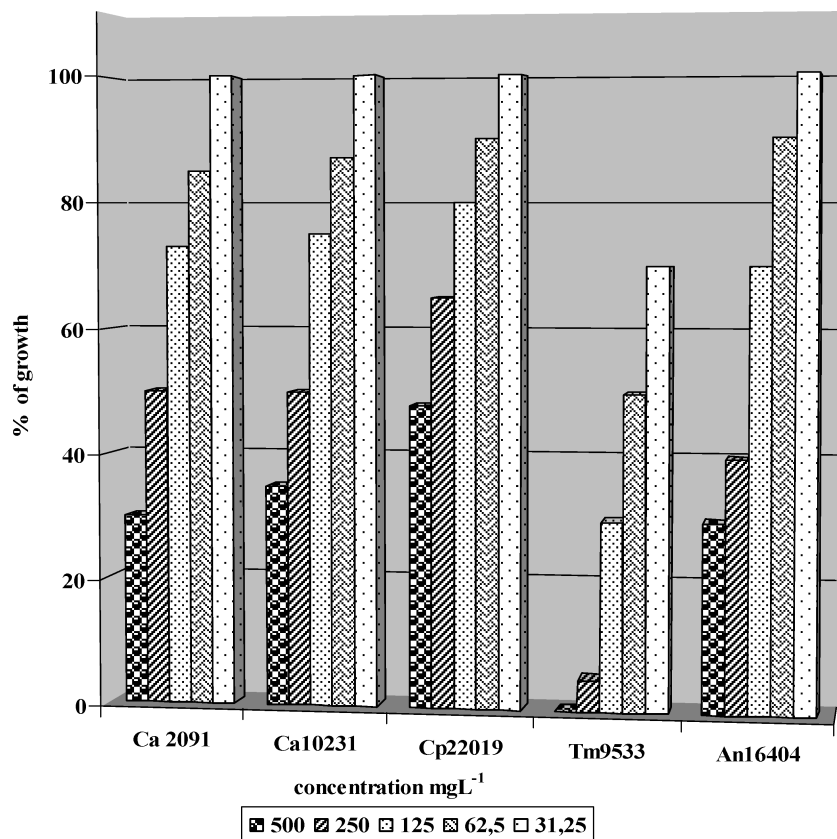


FIGURE 1 The influence of compound **3** on fungi growth (Ca – *Candida albicans*, Cp – *Candida parapsilosis*, Tm – *Trichophyton menthagrophytes*, An – *Aspergillus niger*).

EXPERIMENTAL

Chemistry

Melting points were determined in Fisher-Johns blocs and are presented without any corrections. IR spectra were recorded in KBr using Specord IR-75 spectrophotometer. The ¹H NMR spectra were recorded on a Bruker Avance 300 in DMSO-d₆ with TMS as internal standard. Chemicals were purchased from Lancaster or Merck Co. and used without further purification. Purity was checked by TLC on Merck Co. plates using aluminium oxide 60 F₂₅₄ in a CHCl₃/C₂H₅OH (10:1) solvent system with UV visualization.

1-[(4-Nitroimidazol-1-yl)acetyl]-4-substituted thiosemicarbazide 2–6

0.01 Mol of hydrazide of (4-nitroimidazol-1-yl)acetic acid (1) and 0.01 mol of appropriate isothiocyanate were heated at the 60–70°C for 8 h. The product was washed with Et₂O to remove the unreacted isothiocyanate, dried, and crystallized from EtOH (70–78%).

1-[(4-Nitroimidazol-1-yl)acetyl]-4-phenyl-thiosemicarbazide 2: mp 140–142°C. IR (KBr) cm⁻¹: 3200, 3060, 2980, 1460, 1700, 1552, 1210. ¹H-NMR (DMSO-d₆) δ: 4.95(2H, s), 7.12 (5H, m), 7.76 (1H, m), 8.28 (1H, m), 9.41 (1H, s), 9.68 (1H, s), 10.48 (1H, s). Anal. Calcd for C₁₂H₁₂N₆O₃S: 320.328, C, 44.99; H, 3.78; N, 26.24. Found: C, 44.92; H, 3.71; N, 26.33.

1-[(4-Nitroimidazol-1-yl)acetyl]-4-(4-tolyl)-thiosemicarbazide 3: mp 178–180°C. IR (KBr) cm⁻¹: 3210, 3071, 2950, 1450, 1710, 1556, 1200. ¹H-NMR (DMSO-d₆) δ: 2.23 (3H, s), 4.94 (2H, s), 7.08 (4H, m), 7.79 (1H, s), 8.29 (1H, m), 9.59 (2H, m), 10.43 (1H, s). Anal. Calcd for C₁₃H₁₄N₆O₃S: 334.355 C, 46.69; H, 4.22; N, 25.14. Found: C, 46.32; H, 4.21; N, 25.11.

4-(4-Metoxyphenyl)-1-[(4-nitroimidazol-1-yl)acetyl]thiosemicarbazide 4: mp 192–194°C. IR (KBr) cm⁻¹: 3200, 3070, 2980, 1445, 1700, 1548, 1195. ¹H-NMR (DMSO-d₆) δ: 3.75 (3H, s), 4.94 (2H, s), 6.88 (4H, m), 7.78 (1H, s), 8.28 (1H, m), 9.56 (1H, s), 9.65(1H, s), 10.44(1H, s). Anal. Calcd for C₁₃H₁₄N₆O₄S: 350.354 C, 44.56; H, 4.03; N, 23.99. Found: C, 44.51; H, 4.02; N, 23.90.

4-(4-Bromophenyl)-1-[(4-nitroimidazol-1-yl)acetyl]thiosemicarbazide 5: mp 213–215°C. IR (KBr) cm⁻¹: 3230, 3075, 2940, 1455, 1690, 1545, 1200. ¹H-NMR (DMSO-d₆) δ: 4.95 (2H, s), 7.39 (4H, m), 7.78 (1H, m), 8.28 (1H, m), 9.73 (1H, s), 9.86 (1H, s), 10.50 (1H, s). Anal. Calcd for C₁₂H₁₁BrN₆O₃S: 399.224 C, 36.10; H, 2.78; N, 21.05. Found: C, 36.10; H, 2.76; N, 21.00.

4-(4-Iodophenyl)-1-[(4-nitroimidazol-1-yl)acetyl]thiosemicarbazide 6: mp 202–204°C. IR (KBr) cm⁻¹: 3240, 3058, 2940, 1468, 1698, 1551, 1198. ¹H-NMR (DMSO-d₆) δ: 4.95 (2H, s), 7.25 (4H, m), 7.77 (1H, m), 8.28 (1H, m), 9.70 (1H, s), 9.91 (1H, s), 10.49 (1H, s). Anal. Calcd for C₁₂H₁₁IN₆O₃S: 446.225 C, 32.30; H, 2.48; N, 18.83. Found: C, 32.27; H, 2.46; N, 18.82.

3-[(4-Nitroimidazol-1-yl)methyl]-4-substituted-1,2,4-triazoline-5-thione 7–11

General procedure. 0.01 Mol of thiosemicarbazide **2–6** dissolved in 40–50 ml of 2% aqueous NaOH was refluxed for 2 h. After cooling, the solution was neutralized with dilute hydrochloric acid. The precipitate was filtered off and crystallized from EtOH (75–85%).

3-[(4-Nitroimidazol-1-yl)methyl]-4-phenyl-1,2,4-triazoline-5-thione 7. mp 309–310°C. IR (KBr) cm^{-1} : 3220, 3078, 2976, 1440, 1660, 1230. $^1\text{H-NMR}$ (DMSO-d_6) δ : 5.33 (2H, s), 7.29 (5H, m), 7.63 (1H, d, $J = 1.41$ Hz), 8.15 (1H, d, $J = 1.41$ Hz), 14.02 (1H, s). Anal. Calcd for $\text{C}_{12}\text{H}_{10}\text{N}_6\text{O}_2\text{S}$: 302.313 C, 47.67; H, 3.33; N, 27.80. Found: C, 47.65; H, 3.30; N, 27.79.

3-[(4-Nitroimidazol-1-yl)methyl]-4-(4-tolyl)-1,2,4-triazoline-5-thione 8. mp 292–293°C. IR (KBr) cm^{-1} : 3225, 3075, 2948, 1460, 1650, 1220. $^1\text{H-NMR}$ (DMSO-d_6) δ : 2.50 (3H, s), 5.31 (2H, s), 7.28, 7.36 (4H, dd, $J = 8.3$ Hz), 7.65 (1H, d, $J = 1.39$ Hz), 8.15 (1H, d, $J = 1.39$ Hz), 13.98 (1H, s). Anal. Calcd for $\text{C}_{13}\text{H}_{12}\text{N}_6\text{O}_2\text{S}$: 316.34 C, 49.35; H, 3.82; N, 26.57. Found: C, 49.33; H, 3.74; N, 26.55.

4-(4-Methoxyphenyl)-3-[(4-nitroimidazol-1-yl)methyl]-1,2,4-triazoline-5-thione 9. The synthesis and characterization data were published previously.¹⁵

4-(4-Bromophenyl)-3-[(4-nitroimidazol-1-yl)methyl]-1,2,4-triazoline-5-thione 10. mp 312–314°C. IR (KBr) cm^{-1} : 3240, 3070, 2953, 1432, 1678, 1220. $^1\text{H-NMR}$ (DMSO-d_6) δ : 5.39 (2H, s), 7.43 (2H, d, $J = 8.58$ Hz), 7.78 (2H, d, $J = 8.58$ Hz), 7.72 (1H, s), 8.23 (1H, s), 13.10 (1H, s). Anal. Calcd for $\text{C}_{12}\text{H}_9\text{BrN}_6\text{O}_2\text{S}$: 381.209 C, 37.81; H, 2.38; N, 22.05. Found: C, 37.80; H, 2.29; N, 22.02.

4-(4-Iodophenyl)-3-[(4-Nitroimidazol-1-yl)methyl]-1,2,4-triazoline-5-thione 11. mp 305°C. IR (KBr) cm^{-1} : 3220, 3080, 2957, 1433, 1640, 1230. $^1\text{H-NMR}$ (DMSO-d_6) δ : 5.33 (2H, s), 7.23; 7.93 (4H, dd, $J = 8.58$ Hz), 7.67 (1H, d, $J = 1.39$ Hz), 8.18 (1H, d, $J = 1.39$ Hz), 13.93 (1H, s). Anal. Calcd for $\text{C}_{12}\text{H}_9\text{IN}_6\text{O}_2\text{S}$: 428.21 C, 33.66; H, 2.12; N, 19.63. Found: C, 33.52; H, 2.11; N, 19.64.

Microbiology

The newly synthesized compounds **2–5**, **8**, and **10** were screened for their antimicrobial activity against nine reference strains of aerobic bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12 228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus*

ATCC 10876, *Micrococcus luteus* ATCC 10240, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 12453, *Klebsiella pneumoniae* ATCC 13883, and *Pseudomonas aeruginosa* ATCC 9027) and for five reference strains of fungi (*Candida albicans* ATCC 2091, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, *Trichophyton menthagrophytes* ATCC 9533, and *Aspergillus niger* ATCC 16404). The inoculum density was adjusted to 0.5 McFarland standard (150×10^6 cfu/mL) with sterile saline (0.85% NaCl) and then the suspensions were diluted 1:10 in Mueller-Hinton broth (for bacteria) or Mueller-Hinton broth with 2% glucose (for fungi). All stock solutions of the assayed compounds were prepared in dimethyl sulfoxide (DMSO) with distilled water (1:1). It was found that DMSO at the final concentration in the medium had no influence on growth of the tested microorganisms.

In the first step, all of these compounds were examined in vitro for antibacterial activity using an agar dilution method with Mueller-Hinton medium containing from 31.25 to 500 mg L⁻¹ of the tested compounds and subsequently, for potentially active **2**, **3**, and **10** compounds, spectrophotometrically (OD₆₀₀) by a dilution method with Mueller-Hinton broth containing from 7.81 to 500 mg L⁻¹ of the compounds. The incubation was carried out at 37°C for 18 h.

Antifungal activity was examined using an agar dilution method. Microbial suspensions were put onto Mueller-Hinton agar with 2% glucose buffered at pH 5.6 containing several final concentrations of the tested compounds (31.25–500 mg L⁻¹). The incubation was carried out at 30°C for 24 and 48 h for *Candida* spp. and for 72–96 h for *Aspergillus niger* ATCC 16404 and *T. menthagrophytes* ATCC 9533, depending on the growth in control plates.

Microbial susceptibility testing was performed according to CLSI guideline.¹⁸

The MIC (minimal inhibitory concentration) values were defined as the lowest concentration of the compound required for 100% inhibition of the visible growth of the tested bacteria or prominent growth inhibition (approximately 80% inhibition compared to the growth in the control plates) of fungi.

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