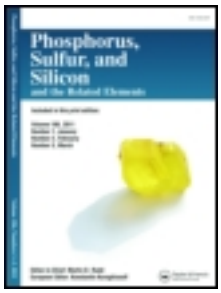

Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:
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Synthesis and antibacterial activity of some new derivatives of thiosemicarbazide and 1,2,4-triazole

Monika Wujec [a], Anna Pachuta-Stec [a], Joanna Stefańska [b], Edyta Kuśmierz [a] & Agata Siwek [a]

[a] Department of Organic Chemistry, Medical University, 4a Chodzki Str., 20-093, Lublin, Poland

[b] Department of Pharmaceutical Microbiology, Medical University, Warsaw, Poland

Accepted author version posted online: 02 Jan 2013.

To cite this article: Monika Wujec , Anna Pachuta-Stec , Joanna Stefańska , Edyta Kuśmierz & Agata Siwek (2013): Synthesis and antibacterial activity of some new derivatives of thiosemicarbazide and 1,2,4-triazole, Phosphorus, Sulfur, and Silicon and the Related Elements, DOI:10.1080/10426507.2012.757612

To link to this article: http://dx.doi.org/10.1080/10426507.2012.757612

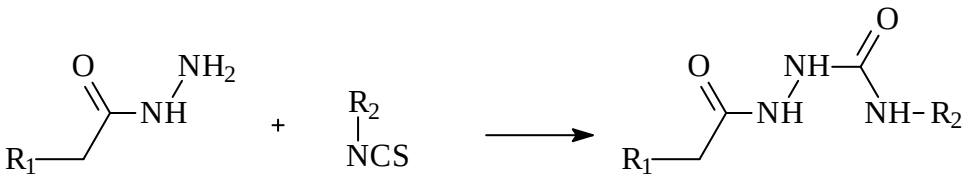
Synthesis and antibacterial activity of some new derivatives of thiosemicarbazide and 1,2,4-triazole.

[a]Monika Wujec*, [a]Anna Pachuta-Stec, [b]Joanna Stefańska, [a]Edyta Kuśmierz, [a]Agata Siwek

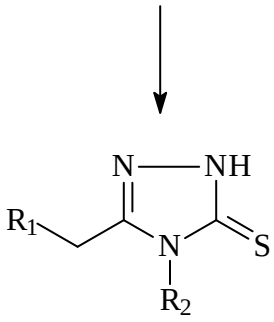
[a]Department of Organic Chemistry, Medical University, 4a Chodzki Str., 20-093 Lublin, Poland,

[b]Department of Pharmaceutical Microbiology, Medical University, Warsaw, Poland

e-mail: monika.wujec@umlub.pl



1,2 **3-18**



19-34

$R_1 = C_6H_5, C_6H_{11}$

$R_2 = C_6H_5, 2\text{-}BrC_6H_4, 4\text{-}BrC_6H_4, 2\text{-}FC_6H_4, 4\text{-}FC_6H_4, 2\text{-}CH_3C_6H_4, 4\text{-}CH_3C_6H_4, C_2H_5$

Abstract: In the reaction of the hydrazides of cyclohexylacetic acid **1** and phenylacetic acid **2** with isothiocyanates, the respective thiosemicarbazide derivatives **3-18** were obtained. Further

cyclization with 2% NaOH led to the formation of 5-(cyclohexylmethyl/benzyl)-4-substituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **19-34**. The structures of all new products were confirmed by analytical and spectroscopic methods. All compounds were screened for their *in vitro* activity against some species of bacteria and fungi.

Keywords: Thiosemicarbazides, 1,2,4-triazoles, isothiocyanates, antimicrobial activity.

INTRODUCTION

Infectious diseases and bacterial infections - despite enormous progress, both in their treatment and prevention - are an increasingly serious medical problem. Practicing physicians are instantly faced with new, more dangerous strains of bacteria, which is, among others, a result of natural selection of bacteria resistant to antibiotics. Therefore many research centers are currently involved in the exploration of new antibacterial and antifungal agents. Our team has been working for many years with synthesis of derivatives of acetic hydrazides: thio/semicarbazide, oxo-/mercapto-triazole and we are also still looking for a relationship between their molecular structure and bioactivity. Where does our interest in these compounds come from? Both derivatives of thio/semicarbazide and triazole have been reported to exhibit antimicrobial, fungicidal, anti-inflammatory, antiparasitic, insecticidal, herbicidal, antiviral, antitumor, anticonvulsant, antidepressant, hypotensive effects and plant growth regulatory activities.[1-18] Additionally, the triazole ring has been incorporated into a wide variety of important drugs: Ribavirin, Rizatriptan, Letrozole, Anastrozole and many antifungal drugs: Fluconazole, Itraconazole, Posaconazole.

In continuation of our study on the synthesis of biologically active compounds, in this paper we present the synthesis and antimicrobial activity of two series of the thiosemicarbazide and triazole derivatives.

RESULTS AND DISCUSSION

Cyclohexyl acetic acid hydrazide 1 and phenylacetic acid hydrazide 2 were used as starting materials, which were obtained by the reaction of ethyl ester of the appropriate acids with 80% hydrazine hydrate[18,19]. New thiosemicarbazide derivatives **3-18** were obtained by the reaction of **1** and **2** with isothiocyanates. The conditions of the reaction were established experimentally. Two of thiosemicarbazide of cyclohexylacetic acid (with bromo- and fluoro substituent in position 2 in phenyl ring) were obtained during heating under reflux for 2 h in ethanol. We tried to use the same method for all compounds, however without success. The rest of thiosemicarbazide derivatives were obtained by heating on the oil bath for 8 h.

The thiosemicarbazides **3-18** were subjected to cyclization in a 2% aqueous solution of sodium hydroxide yielding the corresponding 5-(cyclohexylmethyl/benzyl)-4-substituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **19-34**. The reactions were performed according to Scheme 1.

[Insert Scheme 1]

The structures of the obtained products **3-34** were confirmed by elemental analysis as well as by the ^{1}H NMR spectra. The ^{1}H NMR spectra of the thiosemicarbazide derivatives **3-18** showed the signals of the proton linked to nitrogen atoms at 7.78-10.32 ppm. In the respective triazoles **19-34**, the signal of the NH proton was observed in the range of 13.47-13.97 ppm. All obtained triazoles are present in the thione form according their NMR spectra (absence of a signal of the

proton linked to sulfur). Substituents and corresponding yields of the intermediates **3**-**18** as well as the products **19**-**34** are collected in Tables 1 and 2, respectively.

[Insert Table 1]

[Insert Table 2]

Biological Activity

The *in vitro* antimicrobial activities of all synthesized compounds at concentrations ranging from 400 to 6.25 μg/mL were screened using the agar dilution method against some Gram-negative and Gram-positive reference species of bacteria and a few species of fungi.

Among all tested compounds, only two derivatives of thiosemicarbazide **12** and **16** and one triazole derivative **29** showed antimicrobial activity. Among them, the most active against Gram-positive species was compound **29** (triazole with 4-bromophenyl substituent) with MIC in the range 50-200 μg/mL. Two derivatives of thiosemicarbazide showed inhibitory action against Gram-positive species at somewhat higher concentration (MIC=200-400 μg/mL) (Tables S 1 and S 2, Supplemental Materials). Moreover, compound **16** showed inhibitory effect against Candida albicans ATCC 10231 and ATCC 90028 with MIC = 200 μg/mL and 400 μg/mL, respectively and it was active against most clinical strains of fungi (Table S 3).

In conclusion, our data showed that compounds with phenyl substitution were weakly active whereas the analogs with cyclohexyl ring were inactive. It is possible that, firstly, different conformations of cyclohexyl ring are not suitable for microbiological activity. Secondly, privileged position of substituent in phenyl ring is ortho, but the kind of substituent (electron-donating or electron-withdrawing) in this case is irrelevant.

EXPERIMENTAL

Chemistry

Melting points were determined in Fisher-Johns blocs and presented without any corrections. The ^{1}H NMR spectra were recorded on a Brucker Avance 300 in DMSO-d_6 with TMS as internal standard. Chemicals were purchased from Lancaster or Merck Co. and used without further purification. Purity and progress of reaction were checked by TLC on Merck Co. plates Aluminium oxide 60 F_{254} in a $CHCl_3/C_2H_5OH$ (10:1) solvent system with UV visualization.

1-[(cyclohexyl/phenyl)acetyl]-4-substituted thiosemicarbazide **3-18**

Method for compounds **3**,**5**,**8-10**

The hydrazide of cyclohexylacetic acid (**1**, 0.01 mol) and the appropriate isothiocyanate (0.01 mol) was heated at the 60-70ºC for 8 h. Then the unreacted isothiocyanate was removed by washing with diethyl ether. The obtained crude product was dried and recrystallized to give pure product.

Method for compound 7

The hydrazide of cyclohexylacetic acid (**1,** 0.01 mol) and the 4-fluorophenylisothiocyanate (0.01 mol) was heated at the 100ºC for 10 h. Then the unreacted isothiocyanate was removed by washing with diethyl ether. The obtained crude product was dried and recrystallized to give pure product.

Method for compounds **4**,**6**

The hydrazide of cyclohexylacetic acid (**1,** 0.01 mol) and the (2-bromo/2-fluoro)phenylisothiocyanate (0.01 mol) in 20 mL ethanol was heated under reflux for 2 h. Precipitated compound was filtered off and crystallized from ethanol.

Method for compounds **11-18**

The hydrazide of phenylacetic acid (**2,** 0.01 mol) and the appropriate isothiocyanate (0.01 mol) was heated on oil bath at temperature 40-50ºC for 12 h. The product was washed with Et_2O to remove the unreacted isothiocyanate, dried and crystallized from EtOH.

1-cyclohexylacetyl-4-phenylthiosemicarbazide **3**

mp 170-172ºC. ^{1}H-NMR (DMSO-d_6) δ: 0.91-0.98 (m, 2H, CH_2), 1.13-1.27 (m, 4H, $2CH_2$), 1.62-1.87 (m, 4H, $2CH_2$), 1.89 (s, 1H, CH), 2.04, 2.07 (d, 2H, CH_2, *J*= 6.7 Hz), 7.12-7.45 (m, 5H, arom. benz.), 9.53 (bs, 2H, 2NH), 9.80 (s, 1H, NH). *Anal.* Calcd for $C_{15}H_{21}N_3OS$: 291.41, C, 61.82; H, 7.26; N, 14.42. Found: C, 61.92; H, 7.21; N, 14.43.

1-cyclohexylacetyl-4-(2-bromophenyl)thiosemicarbazide **4**

mp 158-159ºC. ^{1}H-NMR (DMSO-d_6) δ: 0.96-1.13 (m, 2H, CH_2), 1.14-1.29 (m, 4H, $2CH_2$), 1.68-1.79 (m, 4H, $2CH_2$), 1.93 (s, 1H, CH), 2.11, 2.13 (d, 2H, CH_2, *J*= 6.7 Hz), 7.21-7.73 (m, 4H, arom. benz.), 9.48 (s, 2H, 2NH), 9.98 (s, 1H, NH). *Anal.* Calcd for $C_{15}H_{20}BrN_3OS$: 370.31, C, 48.65; H, 5.44; N, 11.35. Found: C, 48.62; H, 5.41; N, 11.41.

1-cyclohexylacetyl-4-(4-bromophenyl)thiosemicarbazide **5**

mp 182-183ºC. ^{1}H-NMR (DMSO-d_6) δ: 0.87-1.03 (m, 2H, CH_2), 1.05-1.27 (m, 4H, $2CH_2$), 1.62-1.72 (m, 4H, $2CH_2$), 1.90(s, 1H, CH), 2.04, 2.06 (d, 2H, CH_2, *J*= 6.6 Hz), 7.42-7.52 (m, 4H, arom. benz.), 9.63 (bs, 2H, 2NH), 9.81 (s, 1H, NH) (Figure S1). *Anal.* Calcd for $C_{15}H_{20}BrN_3OS$: 370.31, C, 48.65; H, 5.44; N, 11.35. Found: C, 48.64; H, 5.41; N, 11.38.

1-cyclohexylacetyl-4-(2-fluorophenyl)thiosemicarbazide **6**

mp 123-125ºC. ^{1}H-NMR (DMSO-d_6) δ: 0.87-1.09 (m, 2H, CH_2), 1.13-1.27 (m, 2H, CH_2), 1.30 (s, 1H, CH), 1.62-1.72 (m, 6H, $3CH_2$), 2.04, 2.06 (d, 2H, CH_2, *J*= 6.6 Hz), 7.14-7.37 (m, 4H,

arom. benz.), 9.30, 9.69, 9.88 (3s, 3H, 3NH). *Anal.* Calcd for $C_{15}H_{20}FN_3OS$: 309.40, C, 58.23; H, 6.52; N, 13.58. Found: C, 58.25; H, 6.51; N, 13.59.

1-cyclohexylacetyl-4-(4-fluorophenyl)thiosemicarbazide **7**

mp 123-125ºC. ^{1}H-NMR (DMSO-d_6) δ: 1.04-1.24 (m, 6H, $3CH_2$), 1.68-1.73 (m, 4H, $2CH_2$), 1.78 (s, 1H, CH), 2.10, 2.12 (d, 2H, CH_2, *J*= 6.6 Hz), 7.18-7.50 (m, 4H, arom. benz.), 9.35, 9.76, 9.98 (3s, 3H, 3NH). *Anal.* Calcd for $C_{15}H_{20}FN_3OS$: 309.40, C, 58.23; H, 6.52; N, 13.58. Found: C, 58.23; H, 6.50; N, 13.59.

1-cyclohexylacetyl-4-(2-methylphenyl)thiosemicarbazide **8**

mp 168-170ºC. ^{1}H-NMR (DMSO-d_6) δ: 0.89-1.11 (m, 2H, CH_2), 1.13-1.27 (m, 2H, CH_2), 1.30 (s, 1H, CH), 1.62-1.72 (m, 6H, $3CH_2$), 2.04, 2.06 (d, 2H, CH_2, *J*= 6.6 Hz), 2.32 (, 3H, CH_3), 7.15-7.42 (m, 4H, arom. benz.), 8.87, 9.52, 9.98 (3s, 3H, 3NH). *Anal.* Calcd for $C_{16}H_{23}N_3OS$: 359.44, C, 62.92; H, 7.59; N, 13.76. Found: C, 62.93; H, 7.51; N, 13.77.

1-cyclohexylacetyl-4-(4-methylphenyl)thiosemicarbazide **9**

mp 158-160ºC. ^{1}H-NMR (DMSO-d_6) δ: 0.80-0.98 (m, 2H, CH_2), 1.03-1.26 (m, 2H, CH_2), 1.30 (s, 1H, CH), 1.61-1.89 (m, 6H, $3CH_2$), 2.03, 2.06 (d, 2H, CH_2, *J*= 6.6 Hz), 2.27 (, 3H, CH_3), 7.10, 7.13, 7.28, 7.31 (dd, 4H, arom. benz. *J*= 7.7 Hz), 8.89, 9.46, 9.77 (3s, 3H, 3NH). *Anal.* Calcd for $C_{16}H_{23}N_3OS$: 359.44, C, 62.92; H, 7.59; N, 13.76. Found: C, 62.94; H, 7.56; N, 13.77.

1-cyclohexylacetyl-4-ethylthiosemicarbazide **10**

mp 184-185ºC. ^{1}H-NMR (DMSO-d_6) δ: 0.83-0.96 (m, 2H, CH_2), 1.02, 1.04, 1.07 (t, 3H, CH_3 *J*= 7.4 Hz), 1.13-1.25 (m, 2H, CH_2), 1.27 (s, 1H, CH), 1.62-1.69 (m, 6H, $3CH_2$), 1.99, 2.01 (d, 2H, CH_2, *J*= 6.7 Hz), 3.39, 3.42, 3.44, 3.46 (q, 2H, CH_2), 7.78, 9.03, 9.57 (3s, 3H, 3NH). *Anal.* Calcd for $C_{11}H_{21}N_3OS$: 243.37, C, 54.29; H, 8.70; N, 17.27. Found: C, 54.31; H, 8.69; N, 17.27.

1-phenylacetyl-4-phenylthiosemicarbazide **11**[19]

1-phenylacetyl-4-(2-bromophenyl)thiosemicarbazide **12**

mp 149-151ºC.[1]H NMR (DMSO-d_6) δ: 3.59 (s, 2H, CH_2), 7.22-7.73 (m, 9H, $CH_{arom.}$), 9.52, 9.82, 10.32 (3s, 3H, 3NH). *Anal.* Calcd for $C_{15}H_{14}BrN_3OS$: 364.26, C, 49.46; H, 3.87; N, 11.54. Found: C, 49.51; H, 3.69; N, 11.27.

1-phenylacetyl-4-(4-bromophenyl)thiosemicarbazide **13**[19]

1-phenylacetyl-4-(2-fluorophenyl)thiosemicarbazide **14**

mp 156-158ºC. [1]H NMR (DMSO-d_6) δ: 3.57 (s, 2H, CH_2), 7.21-7.43 (m, 9H, $CH_{arom.}$), 9.58, 9.83, 10.29 (3s, 3H, 3NH). *Anal.* Calcd for $C_{15}H_{14}FN_3OS$: 303.35, C, 59.39; H, 4.65; N, 13.85. Found: C, 59.50; H, 4.69; N, 13.86.

1-phenylacetyl-4-(4-fluorophenyl)thiosemicarbazide **15**

mp 158-160ºC. [1]H NMR (DMSO-d_6) δ: 3.58 (s, 2H, CH_2), 7.19-7.50 (m, 9H, $CH_{arom.}$), 9.68, 9.76, 10.22 (3s, 3H, 3NH). *Anal.* Calcd for $C_{15}H_{14}FN_3OS$: 303.35, C, 59.39; H, 4.65; N, 13.85. Found: C, 59.47; H, 4.72; N, 13.85.

1-phenylacetyl-4-(2-methylphenyl)thiosemicarbazide **16** [19]

1-phenylacetyl-4-(4-methylphenyl)thiosemicarbazide **17**

mp 154-156ºC. [1]H NMR (DMSO-d_6) δ: 2.34 (s, 3H, CH_3), 3.58 (s, 2H, CH_2), 7.18-7.42 (m, 9H, $CH_{arom.}$), 9.58, 9.66, 10.19 (3s, 3H, 3NH). *Anal.* Calcd for $C_{16}H_{17}N_3OS$: 299.39, C, 64.19; H, 5.72; N, 14.04. Found: C, 64.20; H, 5.69; N, 13.99.

1-phenylacetyl-4-ethylthiosemicarbazide **18**[19]

5-cyclohexylmethyl/benzyl-4-substituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **19**-**34**

General procedure

Thiosemicarbazide **3**-**18** (0.01 mol) was dissolved in 2% aqueous NaOH (40-50 mL) and refluxed for 2h. After cooling, the solution was filtered and neutralized with dilute hydrochloric acid. The precipitate was filtered off and crystallized from EtOH.

5-cyclohexylmethyl-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **19**: mp 172-174ºC. ^{1}H-NMR (DMSO-d_6) δ: 0.72-0.97 (m, 2H, CH_2), 1.00-1.27 (m, 4H, $2CH_2$), 1.38-1.64 (m, 4H, $2CH_2$), 1.66 (s, 1H, CH), 2.30, 2.33 (d, 2H, CH_2, *J*= 6.7 Hz), 7.36-7.61 (m, 5H, $CH_{arom.}$), 13.70 (s, 1H, NH). *Anal.* Calcd for $C_{15}H_{19}N_3S$: 273.40, C, 65.90; H, 7.00; N, 15.37. Found: C, 65.92; H, 7.01; N, 15.37.

5-cyclohexylmethyl-4-(2-bromophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **20**: mp 215-216ºC. ^{1}H-NMR (DMSO-d_6) δ: 0.90-0.98 (m, 2H, CH_2), 1.14-1.30 (m, 4H, $2CH_2$), 1.69-1.78 (m, 4H, $2CH_2$), 1.90 (s, 1H, CH), 2.11, 2.13 (d, 2H, CH_2, *J*= 6.6 Hz), 7.55-7.95 (m, 4H, arom. benz.), 13.84 (s, 1H, NH). *Anal.* Calcd for $C_{15}H_{18}BrN_3S$: 352.29, C, 51.14; H, 5.15; N, 11.93. Found: C, 51.17; H, 5.21; N, 11.97.

5-cyclohexylmethyl-4-(4-bromophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **21**: mp 128-130ºC. ^{1}H-NMR (DMSO-d_6) δ: 0.74-0.84 (m, 2H, CH_2), 1.04-1.14 (m, 2H, CH_2), 1.24 (s, 1H, CH), 1.39-1.64 (m, 6H, $3CH_2$), 2.27, 2.29 (d, 2H, CH_2, *J*= 6.7 Hz), 7.26, 7,29, 7,69, 7.72 (dd, 4H, CH_{arom} *J*=8.7 Hz), 13.80 (s, 1H, NH) (Figure S2). *Anal.* Calcd for $C_{15}H_{18}BrN_3S$: 352.29, C, 51.14; H, 5.15; N, 11.93. Found: C, 51.14; H, 5.19; N, 11.94.

5-cyclohexylmethyl-4-(2-fluorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **22**: mp 222-224ºC. ^{1}H-NMR (DMSO-d_6) δ: 0.77-0.89 (m, 2H, CH_2), 1.02-1.15 (m, 2H, CH_2), 1.28 (s, 1H, CH), 1.37-1.55 (m, 6H, $3CH_2$), 2.27, 2.30 (d, 2H, CH_2, *J*= 6.7 Hz), 7.39-7,67 (m, 4H, CH_{arom}), 13.82 (s, 1H, NH). *Anal.* Calcd for $C_{15}H_{18}FN_3S$: 291.39, C, 61.83; H, 6.23; N, 14.42. Found: C, 61.85; H, 6.34; N, 14.44.

5-cyclohexylmethyl-4-(4-fluorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **23**: mp 180-182ºC. ^{1}H-NMR (DMSO-d_6) δ: 1.04-1.25 (m, 6H, $3CH_2$), 1.66-1.73 (m, 4H, $2CH_2$), 1.79 (s, 1H, CH), 2.10, 2.12 (d, 2H, CH_2, *J*= 6.6 Hz), 7.46-7.59 (m, 4H, CH_{arom}), 13,89 (s, 1H, NH). *Anal.* Calcd for $C_{15}H_{18}FN_3S$: 291.39, C, 61.83; H, 6.23; N, 14.42. Found: C, 61.83; H, 6.25; N, 14.48.

5-cyclohexylmethyl-4-(2-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **24**: mp 172-174ºC. ^{1}H-NMR (DMSO-d_6) δ: 0.76-0.96 (m, 2H, CH_2), 1.13-1.25 (m, 2H, CH_2), 1.30 (s, 1H, CH), 1.60-1.84 (m, 6H, $3CH_2$), 2.02, 2.04 (d, 2H, CH_2, *J*= 6.6 Hz), 2.50 (s, 3H, CH_3), 7.29-7.57 (m, 4H, arom. benz.), 13,81 (s, 1H, NH). *Anal.* Calcd for $C_{16}H_{21}N_3S$: 287.42, C, 66.86; H, 7.36; N, 14.62. Found: C, 66.83; H, 7.35; N, 14.58.

5-cyclohexylmethyl-4-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **25**: mp 88-92ºC. ^{1}H-NMR (DMSO-d_6) δ: 0.76-0.97 (m, 2H, CH_2), 1.04-1.27 (m, 2H, CH_2), 1.42 (s, 1H, CH), 1.51-1.76 (m, 6H, $3CH_2$), 2.28, 2.31 (d, 2H, CH_2, *J*= 6.7 Hz), 2.39 (s, 3H, CH_3), 7.22, 7.25, 7.35, 7.37 (dd, 4H, CH_{arom} *J*=8.3 Hz), 13,67 (s, 1H, NH). *Anal.* Calcd for $C_{16}H_{21}N_3S$: 287.42, C, 66.86; H, 7.36; N, 14.62. Found: C, 66.88; H, 7.39 N, 14.62.

5-cyclohexylmethyl-4-ethyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **26**: mp 129-132ºC. ^{1}H-NMR (DMSO-d_6) δ: 0.94-1.11 (m, 2H, CH_2), 1.16, 1.19, 1.21 (t, 3H, CH_3 *J*=7.1 Hz), 1.23-1.27 (m, 2H, CH_2), 1.28 (s, 1H, CH), 1.60-1.78 (m, 6H, $3CH_2$), 2.54, 2.56 (d, 2H, CH_2, *J*= 6.7 Hz), 3.91, 3.93,

3.96, 3.98 (q, 2H, CH_2 *J*= 7.1 Hz), 13,47 (s, 1H, NH). *Anal.* Calcd for $C_{11}H_{19}N_3S$: 225.35, C, 58.63; H, 8.50; N, 18.65. Found: C, 58.68; H, 8.49 N, 18.62.

5-benzyl-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **27**[19]

5-benzyl-4-(2-bromophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **28** : mp 230-232ºC.

^{1}H NMR (DMSO-d_6) δ: 3.73 (q, 2H, CH_2 *J* =16.26 Hz), 6.96-7.88 (m, 9H, $CH_{arom.}$), 13.90 (s, 1H, NH). *Anal.* Calcd for $C_{15}H_{12}BrN_3S$: 346.24, C, 52.03; H, 3.49; N, 12.14. Found: C, 52.20; H, 3.69; N, 12.16.

5-benzyl-4-(4-bromophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **29**[19]

5-benzyl-4-(2-fluorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **30**: mp 204-206ºC.

^{1}H NMR (DMSO-d_6) δ: 3.83 (q, 2H, CH_2 *J* =16.00 Hz), 6.91-7.66 (m, 9H, $CH_{arom.}$), 13.97 (s, 1H, NH). *Anal.* Calcd for $C_{15}H_{12}FN_3S$: 285.34, C, 63.14; H, 4.24; N, 14.73. Found: C, 63.15; H, 4.29; N, 14.87.

5-benzyl-4-(4-fluorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **31**: mp 199-201ºC.

^{1}H NMR (DMSO-d_6) δ: 3.92 (s, 2H, CH_2), 6.97-7.43 (m, 9H, $CH_{arom.}$), 13,88 (s, 1H, NH). . *Anal.* Calcd for $C_{15}H_{12}FN_3S$: 285.34, C, 63.14; H, 4.24; N, 14.73. Found: C, 63.25; H, 4.25; N, 14.70.

5-benzyl-4-(2-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **32**[19]

5-benzyl-4-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **33**: mp 182-184ºC.

^{1}H NMR (DMSO-d_6) δ: 2.42 (s, 3H, CH_3), 3.89 (s, 2H, CH_2), 6.99-7.35 (m, 9H, $CH_{arom.}$), 13,79 (s, 1H, NH). . *Anal.* Calcd for $C_{16}H_{15}N_3S$: 281.37, C, 68.30; H, 5.37; N, 14.93. Found: C, 63.19; H, 5.29; N, 14.96.

5-benzyl-4-ethyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **34**[19]

Microbiology

The following microorganisms were used in this study: *Staphyloccocus aureus* NCTC 4163, *S. aureus* ATCC 25923, *S. aureus* ATCC 6538, *S. aureus* ATCC 29213, *.S epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *B. cereus* ATCC 11778, *Micrococcus luteus* ATCC 9341, *M. luteus* ATCC 10240, *Enterococcus. feacalis* ATTC 29212, *E. hirae* ATCC 10541 (as representative examples of Gram-positive bacteria) and *Escherichia coli* ATCC 10538, *E. coli* ATCC 25922, *E. coli* NCTC 8196, *Proteus vulgaris* NCTC 4635, *Pseudomonas aeruginosa* ATCC 15442, *P. aeruginosa* NCTC 6749, *P. aeruginosa* ATCC 27853, *Bordetella bronchiseptica* ATCC 4617 (as representative examples of Gram-negative bacteria) and for 15 strains of fungi (*Candida albicans* ATCC 90028, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, thirteen clinical isolates of *C. albicans* from the Department of Pharmaceutical Microbiology of Warsaw Medical University).

Initially, antibacterial activity was screened on the basis of growth inhibition zone (giz) utilizing the disc diffusion method. For compounds showing the inhibitory effect on the growth of tested bacteria, monitored as an appearance of giz, the minimal inhibitory concentrations (MICs) were determined as the lowest concentration of the compound preventing growth of the tested microorganism using agar dilution method[20-21]. Ciprofloxacin, antibiotic widely used in the treatment of infections diseases, was used as control antimicrobial agent.

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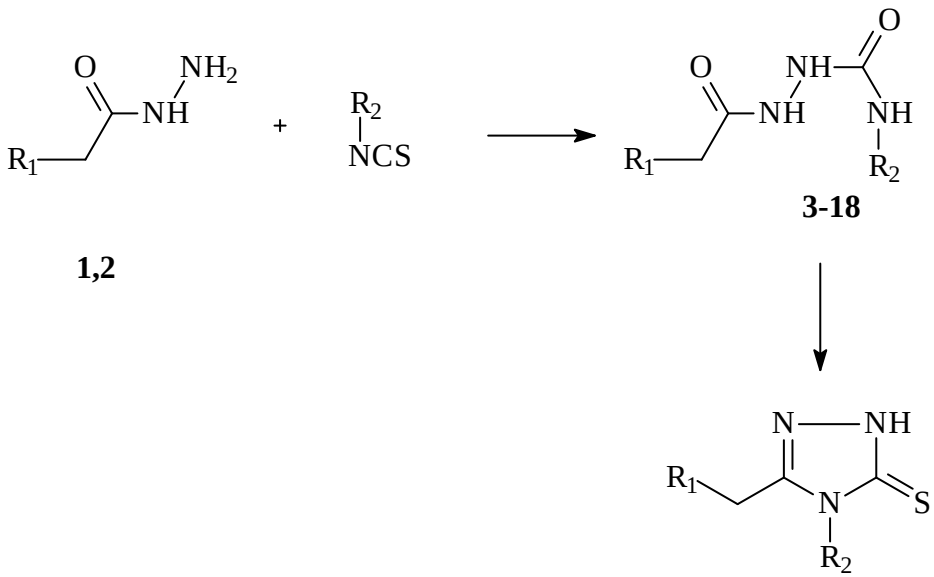
Scheme 1:



R_1 = C_6H_5, C_6H_{11}

R_2 = C_6H_5, 2-BrC_6H_4, 4-BrC_6H_4, 2-FC_6H_4, 4-FC_6H_4, 2-$CH_3C_6H_4$, 4-$CH_3C_6H_4$, C_2H_5

Table 1. Substituents and yields of thiosemicarbazide derivatives

Intermediate			
	R_1	R_2	Yield %
3	C_6H_{11}	C_6H_5	88
4	C_6H_{11}	2-BrC_6H_4	92
5	C_6H_{11}	4-BrC_6H_4	93

6	C_6H_{11}	2-FC_6H_4	94
7	C_6H_{11}	4-FC_6H_4	89
8	C_6H_{11}	2-$CH_3C_6H_4$	78
9	C_6H_{11}	4-$CH_3C_6H_4$	86
10	C_6H_{11}	C_2H_5	80
11	C_6H_5	C_6H_5	95
12	C_6H_5	2-BrC_6H_4	85
13	C_6H_5	4-BrC_6H_4	89
14	C_6H_5	2-FC_6H_4	87
15	C_6H_5	4-FC_6H_4	89
16	C_6H_5	2-$CH_3C_6H_4$	90
17	C_6H_5	4-$CH_3C_6H_4$	93
18	C_6H_5	C_2H_5	93

Table 2. Substituents and yields of triazole derivatives

Product			
	R_1	R_2	Yield %
19	C_6H_{11}	C_6H_5	86
20	C_6H_{11}	2-BrC_6H_4	89
21	C_6H_5	4-BrC_6H_4	87
22	C_6H_5	2-FC_6H_4	90

23	C_6H_{11}	4-FC_6H_4	90
24	C_6H_{11}	2-$CH_3C_6H_4$	86
25	C_6H_{11}	4-$CH_3C_6H_4$	88
26	C_6H_{11}	C_2H_5	76
27	C_6H_5	C_6H_5	84
28	C_6H_5	2-BrC_6H_4	87
29	C_6H_5	4-BrC_6H_4	82
30	C_6H_5	2-FC_6H_4	79
31	C_6H_5	4-FC_6H_4	80
32	C_6H_5	2-$CH_3C_6H_4$	81
33	C_6H_5	4-$CH_3C_6H_4$	83
34	C_6H_5	C_2H_5	84

