Acta Poloniae Pharmaceutica – Drug Research, Vol. 66 No. 1 pp. 73–82, 2009 ISSN 0001-6837
Polish Pharmaceutical Society

SYNTHESIS, LIPOPHILICITY AND ANTIMICROBIAL ACTIVITY OF NEW DERIVATIVES OF THIOSEMICARBAZIDES AND 1,2,4-TRIAZOLINE-5-THIONE

MONIKA WUJEC[1]*, JOANNA STEFAŃSKA[2], AGATA SIWEK[1] and MAŁGORZATA TATARCZAK[3]

[1]Department of Organic Chemistry, Medical University, 6 Staszica St., 20-081 Lublin, Poland.
[2]Department of Pharmaceutical Microbiology, Medical University, 3 Oczki St., Warszawa, Poland.
[3]Department of Inorganic and Analytical Chemistry, Medical University,
6 Staszica St., 20-081 Lublin, Poland

Abstract: In the reaction of hydrazide of 3,4-dimethoxyphenylacetic acid (**1**) with isothiocyanate the respective thiosemicarbazide derivatives (**2**) were obtained. Further cyclization with 2% NaOH led to the formation of 3-[(3,4-dimethoxyphenyl)methyl]-4-substituted-1,2,4-triazoline-5-thiones (**3**). 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 aerobic bacteria and fungi. The lipophilicity of the synthesized molecules was also studied.

Keywords: thiosemicarbazide, 1,2,4-triazole, antimicrobial activity, lipophilicity

The increasing clinical importance of drug-resistant microbial pathogens has lend additional urgency for microbiological and antifungal research. 1,2,4-Triazole and its derivatives represent one of the most biologically active class of compounds possessing a wide spectrum of activities. The 1,2,4-triazole nucleus is associated with diverse pharmacological activities such as antimicrobial, fungicidal, anti-inflammatory, antiparasitic, insecticidal, herbicidal, antiviral, antitumor, anticonvulsant, antidepressant, hypotensive effects and plant growth regulatory activities (1-13). The scientific literature also states that the antibacterial (14, 15) and antiviral (16) activities of thiourea derivatives are due to the presence of the –NH-C(S)-NH- function in the molecule and the changes in this activity depend on the nature of its substituents.

The role of hydrophobic character of a substance is a fundamental physicochemical property that plays a pivotal role in the absorption, distribution, metabolism, and elimination (ADME) of therapeutic drugs. For determination of lipophilicity of biologically active compounds chromatographic techniques are chosen because the behavior and interaction between molecules in chromatographic and biological systems are very similar.

In this paper we describe synthesis of some new thiosemicarbazide and 1,2,4-triazole derivatives and a screen for their *in vitro* activity against some species of aerobic bacteria and fungi. Additionally, the lipophilicity of tested compounds was studied in a hope to provide a deeper insight into the differences in biological activity between them and to suggest synthesis of new, more active thiosemicarbazide and 1,2,4-triazole derivatives.

EXPERIMENTAL

Chemistry

Melting points were determined in Fisher-Johns block and are presented without any corrections. The IR spectra were recorded in KBr discs using Specord IR-75 spectrophotometer. The ^{1}H NMR spectra were recorded on a Brucker Avance 300 in DMSO-d_6 with TMS as an internal standard. Chemicals were purchased from Lancaster or Merck Co. and used without further purification. Purity was checked by TLC on Merck Co. plates Aluminium oxide 60 F_{254} in $CHCl_3$/C_2H_5OH (10 : 1, v/v) solvent system with UV visualization.

1-(3,4-Dimethoxyphenylacetyl)-4-substituted thiosemicarbazides (**2a-2j**)

0.01 mol of hydrazide of 3,4-dimethoxyphenylacetic acid (**1**) and 0.01 mol of respective isothiocyanate were heated at 70-80^{o}C for 10 h (reaction

* Corresponding author: e-mail: monika.wujec@am.lublin.pl

with benzoyl isothiocynate was carried out at room temperature). The product was washed with Et_2O to remove the unreacted isothiocyanate, dried and crystallized from EtOH (67-86%).

4-(4-Tolyl)-1-(3,4-dimethoxyphenylacetyl)thiosemicarbazide (**2a**)

M.p. 162-164°C, yield 82%. IR (cm^{-1}): 3308 NH; 2939, 1516 CH arom.; 1690 C=O; 1460 CH aliph.; 1349 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 2.29 (s, 3H, C**H**$_3$); 3.48 (s, 2H, C**H**$_2$); 3.73 (s, 3H, C**H**$_3$); 3.75 (s, 3H, C**H**$_3$); 6.77-6.95 (m, 3H, arom.); 7.13-7.32 (dd, 4H, arom. *J* = 8.2 Hz); 9.53, 9.57, 10.09 (3s, 3H, 3N**H**). Analysis: Calcd. for $C_{18}H_{21}N_3O_3S$: C 60.15, H 5.89, N 11.69%. Found: C 60.11, H 5.77, N 11.68%.

4-(4-Metoxyphenyl)-1-(3,4-dimethoxyphenylacetyl)thiosemicarbazide (**2b**)

M.p. 170-172°C, yield 80%. IR (cm^{-1}): 3296 NH; 2936, 1516 CH arom.; 1680 C=O; 1466 CH aliph.; 1350 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 3.44 (s, 2H, C**H**$_2$); 3.73 (s, 3H, C**H**$_3$); 3.75 (s, 3H, C**H**$_3$); 3.76 (s, 3H, C**H**$_3$); 6.81-7.30 (m, 7H, arom.); 9.49, 9.54, 10.06 (3s, 3H, 3N**H**). Anaysis: Calcd. for $C_{18}H_{21}N_3O_4S$: C 57.58, H 5.64, N 11.19%. Found: C 57.58, H 5.62, N 11.10%.

4-(4-Bromophenyl)-1-(3,4-dimethoxyphenylacetyl)thiosemicarbazide (**2c**)

M.p. 168-170°C, yield 86%. IR (cm^{-1}): 3311 NH; 2960, 1516 CH arom.; 1690 C=O; 1460 CH aliph.; 1372 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 3.45 (s, 2H, C**H**$_2$); 3.73 (s, 3H, C**H**$_3$); 3.75 (s, 3H, C**H**$_3$); 6.76-6.95 (m, 3H, arom.); 7.43-7.55 (dd, 4H, arom. *J* = 8.7 Hz); 9.72 (s, 2H, 2N**H**); 10.13 (s, 1H, N**H**). Analysis: Calcd. for $C_{17}H_{18}BrN_3O_3S$: C 48.12, H 4.28, N 9.90%. Found: C 48.12, H 4.25, N 9.58%.

4-(2-Fluorophenyl)-1-(3,4-dimethoxyphenylacetyl)thiosemicarbazide (**2d**)

M.p. 175-176°C, yield 86%. IR (cm^{-1}): 3309 NH; 2936, 1516 CH arom.; 1655 C=O; 1466 CH aliph.; 1328 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 3.33 (s, 2H, C**H**$_2$); 3.72 (s, 3H, C**H**$_3$); 3.74 (s, 3H, C**H**$_3$); 6.73-7.27 (m, 7H, arom.); 9.47, 9.77, 10.16 (s, 3H, 3N**H**). Analysis: Calcd. for $C_{17}H_{18}FN_3O_3S$: C 56.19, H 4.99, N 11.56%. Found: C 56.11, H 4.98, N 11.52%.

4-(4-Fluorophenyl)-1-(3,4-dimethoxyphenylacetyl)thiosemicarbazide (**2e**)

M.p. 110-113°C, yield 80%. IR (cm^{-1}): 3300 NH; 2950, 1515 CH arom.; 1660 C=O; 1460 CH aliph.; 1330 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 3.44 (s, 2H, C**H**$_2$); 3.72 (s, 3H, C**H**$_3$); 3.74 (s, 3H, C**H**$_3$); 6.80-7.53 (m, 7H, arom.); 9.63, 9.66, 10.10 (s, 3H, 3N**H**). Analysis: Calcd. for $C_{17}H_{18}FN_3O_3S$: C 56.19, H 4.99, N 11.56%. Found: C 56.17, H 4.97, N 11.52%.

4-(2-Iodophenyl)-1-(3,4-dimethoxyphenylacetyl) thiosemicarbazide (**2f**)

M.p. 153-155°C, yield 75%. IR (cm^{-1}): 3300 NH; 2950, 1517 CH arom.; 1680 C=O; 1407 CH aliph.; 1370 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 3.44 (s, 2H, C**H**$_2$); 3.71 (s, 3H, C**H**$_3$); 3.74 (s, 3H, C**H**$_3$); 6.76-7.89 (m, 7H, arom.); 9.41, 9.69, 10.17 (3s, 3H, 3N**H**). Analysis: Calcd. for $C_{17}H_{18}IN_3O_3S$: C 43.32, H 3.85, N 8.92%. Found: C 43.54, H 3.83, N 8.91%.

4-(4-Iodophenyl)-1-(3,4-dimethoxyphenylacetyl) thiosemicarbazide (**2g**)

M.p. 185-186°C, yield 80%. IR (cm^{-1}): 3307 NH; 2970, 1517 CH arom.; 1681 C=O; 1467 CH aliph.; 1371 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 3.45 (s, 2H, C**H**$_2$); 3.73 (s, 3H, C**H**$_3$); 3.74 (s, 3H, C**H**$_3$); 6.75-6.94 (m, 3H, arom.); 7.30-7.70 (dd, 4H, arom. *J* = 8.5 Hz); 9.70 (s, 2H, 2N**H**); 10.12 (s, 1H, N**H**). Analysis: Calcd. for $C_{17}H_{18}IN_3O_3S$: C 43.32, H 3.85, N 8.92%. Found: C 43.60, H 3.80, N 8.92%.

4-Ethyl-1-(3,4-dimethoxyphenylacetyl)thiosemicarbazide (**2h**)

M.p. 80-83°C, yield 67%. IR (cm^{-1}): 3300 NH; 2970, 1515 CH arom.; 1671 C=O; 1460 CH aliph.; 1330 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 1.03-1.08 (t, 3H, C**H**$_3$ *J* = 7.1 Hz); 3.39 (s, 2H, C**H**$_2$); 3.42-3.49 (q, 2H, C**H**$_2$ *J* = 6.6 Hz); 3.73 (s, 3H, C**H**$_3$); 3.74 (s, 3H, C**H**$_3$); 6.68-6.90 (m, 3H, arom.); 7.86-7.88 (t, 1H, NH *J* = 5.5 Hz); 9.14, 9.85 (2s, 2H, 2N**H**). Analysis: Calcd. for $C_{13}H_{19}N_3O_3S$: C 52.51, H 6.44, N 14.13%. Found: C 52.60, H 6.40, N 14.02%.

4-Butyl-1-(3,4-dimethoxyphenylacetyl)thiosemicarbazide (**2i**)

M.p. 125-127°C, yield 71%. IR (cm^{-1}): 3302 NH; 2960, 1515 CH arom.; 1670 C=O; 1460 CH aliph.; 1337 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 0.84-0.89 (t, 3H, C**H**$_3$ *J* = 7.2 Hz); 1.21-1.28 (m, 2H, C**H**$_2$); 1.43-1.48 (m, 2H, C**H**$_2$); 3.38 (s, 2H, C**H**$_2$); 3.42-3.45 (m, 2H, C**H**$_2$); 3.72-3 (s, 3H, C**H**$_3$); 3.74 (s, 3H, C**H**$_3$); 6.71-6.93 (m, 3H, arom.); 7.79, 9.13, 9.84 (3s, 3H, 3N**H**). Analysis: Calcd. for $C_{15}H_{23}N_3O_3S$: C 55.36, H 7.12, N 12.91%. Found: C 55.50, H 7.20, N 13.02%.

4-Cyclohexyl-1-(3,4-dimethoxyphenylacetyl)thiosemicarbazide (**2j**)

M.p. 188-189°C, yield 70%. IR (cm^{-1}): 3314 NH; 2960, 1515 CH arom.; 1672 C=O; 1460 CH aliph.; 1332 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 1.04-1.78 (m, 10H, 5C**H**$_2$); 3.43 (s, 2H, C**H**$_2$); 3.73 (s, 3H, C**H**$_3$); 3.75 (s, 3H, C**H**$_3$); 4.05 (s, 1H, C**H**); 6.72-6.95 (m, 3H, arom.); 7.23, 9.15, 9.88 (s, 3H, 3N**H**). Analysis: Calcd. for $C_{17}H_{25}N_3O_3S$: C 58.09, H 7.17, N 11.96%. Found: C 58.08, H 7.20, N 11.97%.

3-(3,4-Dimethoxyphenylmethyl)-4-substituted-1,2,4-triazoline-5-thiones (**3a-3j**)

General procedure

0.01 mol of thiosemicarbazide (**2a-2j**) 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 (65-90%).

4-(4-Tolyl)-3-(3,4-dimethoxyphenylmethyl)-1,2,4-triazoline-5-thione (**3a**)

M.p. 158-159°C, yield 79%. IR (cm^{-1}): 3300 NH; 3060, 1513 CH arom.; 1578 C=N; 1440 CH aliph.; 1335 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 2.37 (s, 3H, C**H**$_3$); 3.58 (s, 3H, C**H**$_3$); 3.68 (s, 3H, C**H**$_3$); 3.77 (s, 2H, C**H**$_2$); 6.40-6.80 (m, 3H, arom); 7.09-7.31 (dd, 4H, arom. *J* = 8.1 Hz); 13.72 (s, 1H, N**H**). Analysis: Calcd. for $C_{18}H_{19}N_3O_2S$: C 63.32, H 5.61, N 12.31%. Found: C 63.23, H 5.58, N 12.10%.

4-(4-Methoxyphenyl)-3-(3,4-dimethoxyphenylmethyl)-1,2,4-triazoline-5-thione (**3b**)

M.p. 120-124°C, yield 74%. IR (cm^{-1}): 3310 NH; 2960, 1520 CH arom.; 1570 C=N; 1437 CH aliph.; 1320 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 3.59 (s, 3H, C**H**$_3$); 3.68 (s, 3H, C**H**$_3$); 3.76 (s, 2H, C**H**$_2$); 3.81 (s, 3H, C**H**$_3$); 6.42-6.80 (m, 3H, arom.); 7.01-7.15 (dd, 4H, arom. *J* = 8.9 Hz); 13.71 (s, 1H, NH). Analysis: Calcd. for $C_{18}H_{19}N_3O_3S$: C 60.49, H 5.36, N 11.76%. Found: C 60.41, H 5.35, N 11.70%.

4-(4-Bromophenyl)-3-(3,4-dimethoxyphenylmethyl)-1,2,4-triazoline-5-thione (**3c**)

M.p. 215-218°C, yield 86%. IR (cm^{-1}): 3222 NH; 3008, 1515 CH arom.; 1597 C=N; 1468 CH aliph.; 1343 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 3.59 (s, 3H, C**H**$_3$); 3.68 (s, 3H, C**H**$_3$); 3.80 (s, 2H, C**H**$_2$); 6.42-6.79 (m, 3H, arom.); 7.19-7.71 (dd, 4H, arom. *J* = 8.7 Hz); 13.79 (s, 1H, N**H**). Analysis: Calcd. for $C_{17}H_{16}BrN_3O_2S$: C 50.25, H 3.97, N 10.34%. Found: C 50.26, H 3.80, N 10.32%.

4-(2-Fluorophenyl)-3-(3,4-dimethoxyphenylmethyl)-1,2,4-triazoline-5-thione (**3d**)

M.p. 196-198°C, yield 90%. IR (cm^{-1}): 3501 NH; 2928, 1515 CH arom.; 1573 C=N; 1463 CH aliph.; 1330 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 3.57 (s, 3H, C**H**$_3$); 3.68 (s, 3H, C**H**$_3$); 3.79-3.81 (d, 2H, C**H**$_2$ *J* = 5.5 Hz); 6.38-6.76 (m, 3H, arom.); 7.31-7.61 (m, 4H, arom.); 13.86 (s, 1H, N**H**). Analysis: Calcd. for $C_{17}H_{16}FN_3O_2S$: C 59.12, H 4.67, N 12.17%. Found: C 59.20, H 4.60, N 12.20%.

4-(4-Fluorophenyl)-3-(3,4-dimethoxyphenylmethyl)-1,2,4-triazoline-5-thione (**3e**)

M.p. 225-226°C, yield 88%. IR (cm^{-1}): 3433 NH; 2931, 1515 CH arom.; 1573 C=N; 1459 CH aliph.; 1328 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 3.60 (s, 3H, C**H**$_3$); 3.68 (s, 3H, C**H**$_3$); 3.78 (s, 2H, C**H**$_2$); 6.44-6.79 (m, 3H, arom); 7.26-7.36 (m, 4H, arom); 13.77 (s, 1H, N**H**). Analysis: Calcd. for $C_{17}H_{16}FN_3O_2S$: C 59.12, H 4.67, N 12.17%. Found: C 59.23, H 4.60, N 12.10%.

4-(2-Iodophenyl)-3-(3,4-dimethoxyphenylmethyl)-1,2,4-triazoline-5-thione (**3f**)

M.p. 182-183°C, yield 68%. IR (cm^{-1}): 3210 NH; 2958, 1516 CH arom.; 1570 C=N; 1465 CH aliph.; 1337 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 3.57 (s, 3H, C**H**$_3$); 3.67 (s, 3H, C**H**$_3$); 3.73 (s, 2H, C**H**$_2$); 6.44-6.79 (m, 3H, arom.); 7.16-8.02 (m, 4H, arom.); 13.79 (s, 1H, N**H**). Analysis: Calcd. for $C_{17}H_{16}IN_3O_2S$: C 45.04, H 3.56, N 9.27%. Found: C 45.30, H 3.60, N 9.26%.

4-(4-Iodophenyl)-3-(3,4-dimethoxyphenylmethyl)-1,2,4-triazoline-5-thione (**3g**)

M.p. 210-212°C, yield 65%. IR (cm^{-1}): 3219 NH; 2960, 1516 CH arom.; 1569 C=N; 1465 CH aliph.; 1339 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 3.59 (s, 3H, C**H**$_3$); 3.69 (s, 3H, C**H**$_3$); 3.80 (s, 2H, C**H**$_2$); 6.40-6.79 (m, 3H, arom.); 7.03-7.86 (dd, 4H, arom. *J* = 8.6 Hz); 13.79 (s, 1H, N**H**). Analysis: Calcd. for $C_{17}H_{16}IN_3O_2S$: C 45.04, H 3.56, N 9.27%. Found: C 45.40, H 3.60, N 9.24%.

4-Ethyl-3-(3,4-dimethoxyphenylmethyl)-1,2,4-triazoline-5-thione (**3h**)

M.p. 178-180°C, yield 71%. IR (cm^{-1}): 3300 NH; 2960, 1513 CH arom.; 1570 C=N; 1465 CH aliph.; 1320 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 0.93-0.98 (t, 3H, C**H**$_3$ *J* = 7.1 Hz); 3.72 (s, 6H, 2C**H**$_3$); 3.85-3.92 (q, 2H, C**H**$_2$ *J* = 7.1 Hz); 4.03 (s, 2H, C**H**$_2$); 6.73-6.92 (m, 3H, arom.); 13.54 (s, 1H, N**H**). Analysis: Calcd. for $C_{13}H_{17}N_3O_2S$: C 55.89, H 6.13, N 15.04%. Found: C 55.88, H 6.13, N 15.00%.

4-Butyl-3-(3,4-dimethoxyphenylmethyl)-1,2,4-triazoline-5-thione (**3i**)

M.p. 94-95°C, yield 80%. IR (cm^{-1}): 3300 NH; 2950, 1513 CH arom.; 1570 C=N; 1465 CH aliph.; 1326 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 0.76-0.81 (t, 3H, C**H**$_3$ *J* = 7.1 Hz); 1.16-1.32 (m, 4H, 2C**H**$_2$); 3.34 (s, 2H, C**H**$_2$); 3.72 (s, 6H, 2C**H**$_3$); 3.77-3.82 (m, 2H, C**H**$_2$); 4.03 (s, 2H, C**H**$_2$); 6.73-6.96 (m, 3H, arom.); 13.55 (s, 1H, N**H**). Analysis: Calcd. for $C_{15}H_{21}N_3O_2S$: C 58.61, H 6.89, N 13.67%. Found: C 58.58, H 6.89, N 13.60%.

4-Cyclohexyl-3-(3,4-dimethoxyphenylmethyl)-1,2,4-triazoline-5-thione (**3j**)

M.p. 125-127°C, yield 86%. IR (cm^{-1}): 3301 NH; 2980, 1515 CH arom.; 1573 C=N; 1465 CH aliph.; 1325 C=S. ^{1}H NMR (DMSO-d_6, δ ppm): 1.06-1.73 (m, 10H, 5C**H**$_2$); 3.72 (s, 6H, 2C**H**$_3$); 4.08 (s, 2H, C**H**$_2$); 4.15 (s, 1H, C**H**); 6.70-6.92 (m, 3H, arom.); 13.49 (s, 1H, N**H**). Analysis: Calcd. for $C_{17}H_{23}N_3O_2S$: C 61.23, H 6.95, N 12.60%. Found: C 61.20, H 6.96, N 12.60%.

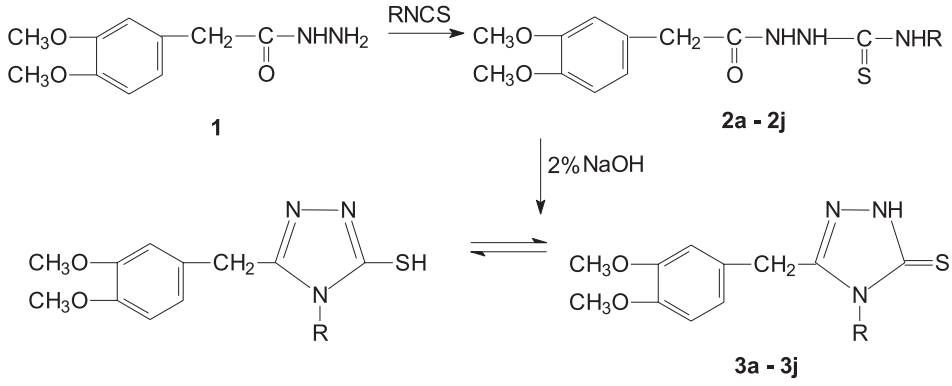
Microbiology

The assessment of the antimicrobial action of the synthesized compounds was performed using the disc-diffusion method and the minimal inhibitory concentration (MIC). Minimum inhibitory concentrations (MICs) were defined as the lowest concentration of the compounds that inhibited visible growth of microorganisms after 18 h incubation at 35°C. Microorganisms used in this study were as follows: Gram-positive bacteria: three strains of *Staphylococcus aureus* (NCTC 4163, ATCC 25923, ATCC 6538), *Bacillus subtilis* ATCC 6633, *Enterococcus hirae* ATCC 10541 and Gram-negative rods: two strains of *Escherichia coli* (ATCC 10538, ATCC 25922), three strains of *Pseudomonas aeruginosa* (ATCC 15442, NCTC 6749, ATCC 27863), *Bordetella bronchiseptica* ATCC 4617. For testing antifungal activities of the compounds *Candida albicans* ATCC 10231 were used. Antimicrobial activity was tested under standard conditions using Mueller-Hinton II agar medium (Becton Dickinson) for bacteria and RPMI agar medium with 2% glucose (Sigma) for yeasts, according to CLSI guidelines (17, 18).

The compounds were dissolved in DMSO. Next, for disc diffusion assay, sterile filter paper discs (9 mm diameter, Whatman No. 3 chromatography paper) were dripped with solutions of tested compound to load 400 μg per disc. Dry discs were placed on the surface of appropriate agar medium. The diameter of growth inhibition zone was read after 18 h of incubation at 35°C. For determination of MICs, concentrations of tested compounds were in the range from 6.25 to 400 μg/mL in a solid medium. The final inoculum of all microorganisms was 10^4 cfu/mL (colony forming units per mL), except the inoculum for *E. hirae* ATCC 10541, which was 10^5 cfu/mL. The results of MICs were read after 18 h of incubation at 35°C. Ciprofloxacin and fluconazole were used as standard antimicrobial powder.

Lipophilicity

Chromatography was performed on 10 cm × 10 cm TLC plates precoated with RP-18 silica F_{254S} gel (Merck, Darmstadt, Germany). Mixtures of methanol, acetonitrile, and water were used as mobile phases. Amounts of organic modifier were in the range 30-70% (v/v) in 10% increments. The compounds were dissolved in methanol (3 mg/mL)



Scheme 1. Synthesis of the compounds

Table 1. Antimicrobial activity of (**2d**) expressed as the growth inhibition zone [giz, mm] and Minimal Inhibitory Concentration (MIC, µg/mL)

Compound / Tested strain	**2d**		Ciprofloxacin		Fluconazole	
	giz	MIC	giz	MIC	giz	MIC
S. aureus ATCC 25923	18	200	26	0.500	nt**	
S. aureus NCTC 4163	13	400	26	0.500	nt	
S. aureus ATCC 29213	13	400	22	0.500	nt	
S. aureus ATCC 6538P	13	400	28	0.500	nt	
B. subtilis ATCC 6633	22	200	40	< 0.125	nt	
E. hirae ATCC 10541	14	400	na	4.000	nt	
E. coli ATCC 10538	na*		34	< 0.125	nt	
E. coli ATCC 25922	na		35	< 0.125	nt	
P. aeruginosa ATCC 15442	na		25	0.500	nt	
P. aeruginosa NCTC 6749	na		26	0.500	nt	
B. bronchiseptica ATCC 4617	na		31	1.000	nt	
C. albicans ATCC 10231	na		nt		22	1.000

*na – no activity in disc diffusion test i. e. a lack of the growth inhibition zone **nt – not tested
Ciprofloxacin (5 mg per disc) Fluconazole (25 µg per disc)

Table 2. The lipophilicity parameters obtained from the linear dependences: $\mathbf{R_M}$ vs.$\boldsymbol{\phi}$ for the investigated compounds

No.	MeOH / H_2O				ACN / H_2O			
	$\mathbf{R_{Mw}}$	***S***	$\boldsymbol{\phi_o}$	R	$\mathbf{R_{Mw}}$	***S***	$\boldsymbol{\phi_o}$	**R**
2a	1,6998	-2,6864	0,6327	0,9799	1,7559	-3,0034	0,5847	0,9895
2b	1,8028	-2,9911	0,6027	0,9550	1,4633	-2,6292	0,5566	0,9900
2c	2,2095	-3,3021	0,6691	0,9869	2,0785	-3,4240	0,6070	0,9855
2d	1,3640	-2,3491	0,5807	0,9638	1,4741	-2,6318	0,5601	0,9937
2e	1,3409	-2,2234	0,6031	0,9649	1,5033	-2,6473	0,5679	0,9907
2f	1,7597	-2,7810	0,6328	0,9760	1,7080	-2,8853	0,5920	0,9832
2g	2,5036	-3,7483	0,6680	0,9831	2,3489	-3,8146	0,6158	0,9664
2h	0,8710	-1,7660	0,4932	0,9967	0,8582	-1,7911	0,4791	0,9762
2i	1,5450	-2,4885	0,6209	0,9920	1,4918	-2,5802	0,5782	0,9937
2j	1,9808	-2,9832	0,6640	0,9780	1,8043	-3,0424	0,5931	0,9861
3a	2,2915	-3,3145	0,6914	0,9933	2,3345	-3,9134	0,5965	0,9446
3b	2,1419	-3,1638	0,6770	0,9780	1,9402	-3,2763	0,5922	0,9680
3c	2,6684	-3,8218	0,6982	0,9930	2,3611	-3,7283	0,6333	0,9834
3d	2,0642	-3,2085	0,6434	0,9753	1,7649	-2,9475	0,5988	0,9791
3e	2,0862	-3,2113	0,6496	0,9980	1,9031	-3,1181	0,6104	0,9713
3f	2,4670	-3,7137	0,6643	0,9769	2,0341	-3,2852	0,6192	0,9827
3g	2,6635	-3,8745	0,6875	0,9851	2,2329	-3,5769	0,6243	0,9911
3h	1,5450	-2,4885	0,6209	0,9920	1,2283	-2,1867	0,5617	0,9446
3i	2,4955	-3,6436	0,6849	0,9812	1,9152	-3,0958	0,6186	0,9813
3j	3,2583	-4,7190	0,6905	0,9588	2,1538	-3,3613	0,6408	0,9842

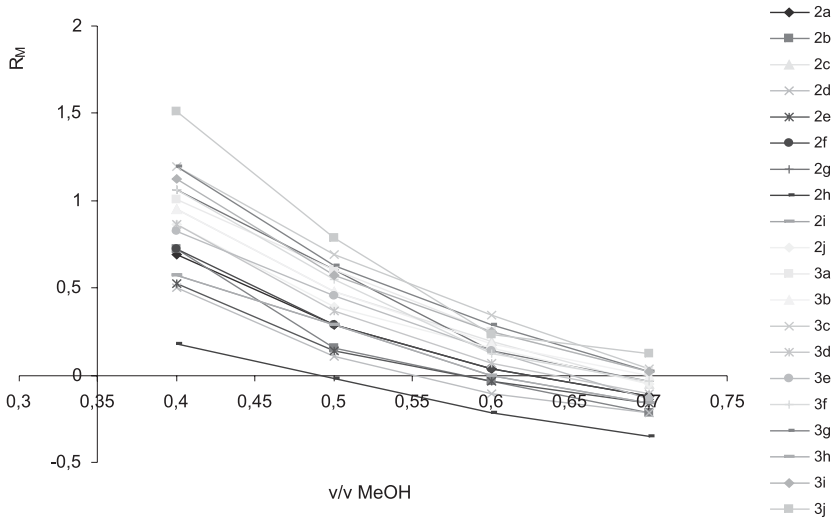


Figure 1. Relationship between R_M values and volume fraction of methanol in the mobile phase.

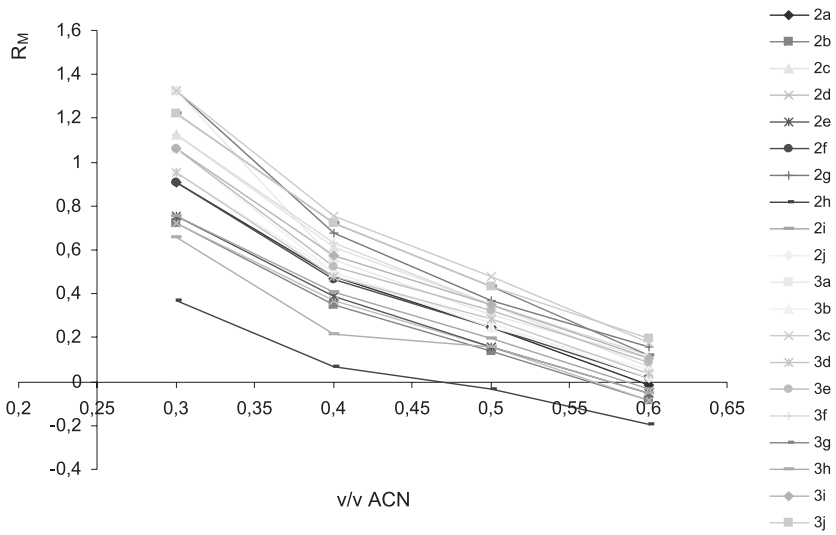


Figure 2. Relationship between R_M values and volume fraction of acetonitrile in the mobile phase.

and samples (10 μm) of the solutions were spotted on the plates. The plates were developed in horizontal Teflon DS chambers (Chromdes, Lublin, Poland) and, after drying, visualized under λ = 254 nm UV light. Chromatograms were developed at 20°C. The volume of the mobile phase was 3 mL in each case. Each experiment was run in quadruplicate. The coefficient of variation values of experimentally obtained R_F were lower than 3%. All reagents were of analytical reagent grade.

RESULTS AND DISCUSSION

The title compounds were synthesized according to a procedure described previously (19) (Scheme 1). For the starting material for synthesis of new derivatives of 1,2,4-triazoline-5-thione we have used 3,4-dimethoxyphenylacetic acid hydrazide (**1**). It was obtained in the reaction of ethyl 3,4-dimethoxyphenylacetate with 80% hydrazine hydrate. New thiosemicarbazide derivatives (**2**)

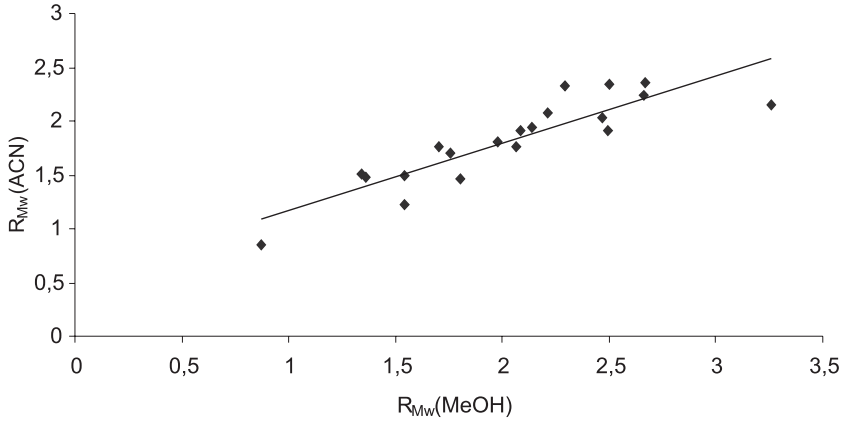


Figure 3. Correlation of R_{Mw} values determined in two elution systems: methanol and acetonitrile.

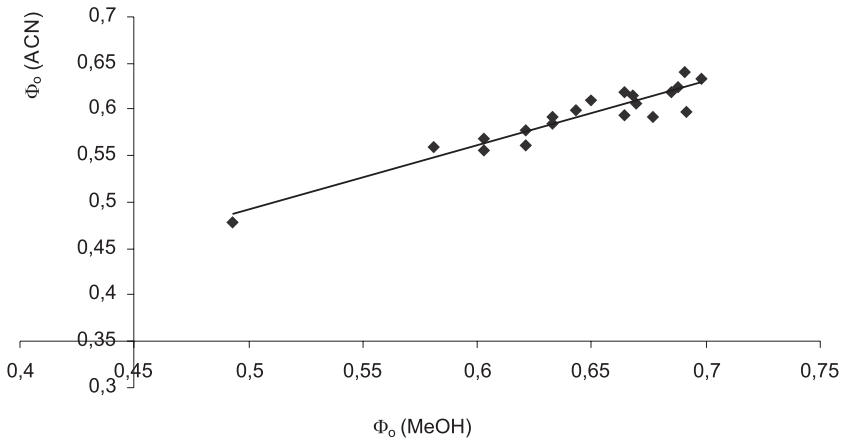


Figure 4. Correlation of Φ_o values determined in two elution systems: methanol and acetonitrile.

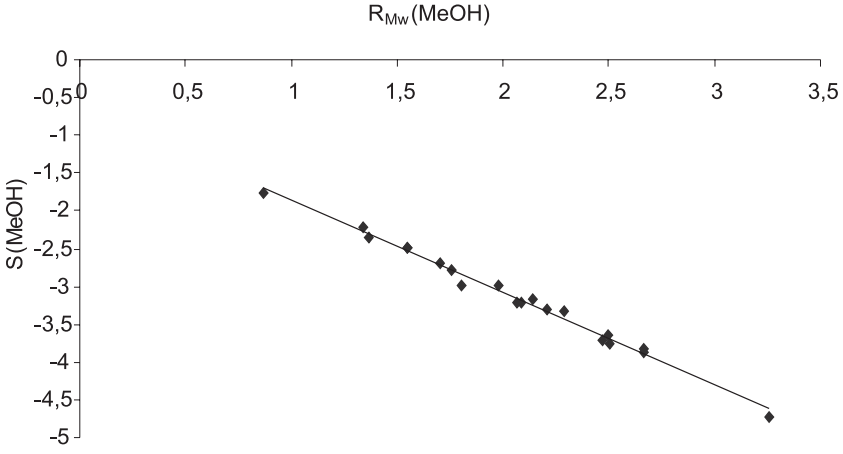


Figure 5. Correlation of S and R_{Mw} values determined in two elution systems: methanol and water.

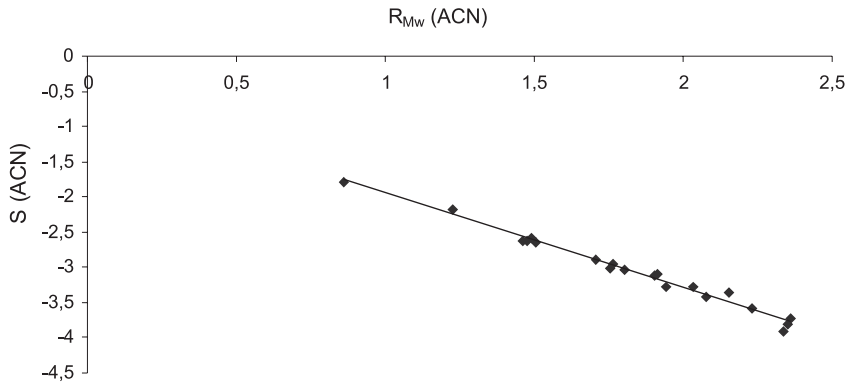


Figure 6. Correlation of *S* and R_{Mw}values determined in two elution systems acetonitrile and water.

were obtained by the reaction of (**1**) with isothiocyanates. The conditions of the reaction were established experimentally. Thiosemicarbazides (**2**) were subjected to cyclization in 2% solution of sodium hydroxide yielding corresponding 3-(3,4-dimethoxyphenylmethyl)-4-substituted-1,2,4-triazoline-5-thione (**3**).

The structure of the obtained products was confirmed by the elemental analysis as well as by the IR and ^{1}H NMR spectra. As indicated in Scheme 1, products of the cyclization can exist in two major tautomeric forms; thiol (the structure on the left) and thione. Compounds (**3**) were present in the solid state in the C=S form as indicated by their IR spectra (absence of absorption in the region of 2500-2600 cm^{-1} for S-H stretching and presence of absorption maxima in the range 1320-1343 cm^{-1}).

The antimicrobial activities of all synthesized compounds were tested against series of Gram-positive bacteria, Gram-negative bacteria and *Candida albicans* ATCC 10231. At first, tests using disc-diffusion method were performed. Compounds that showed activity in these tests were later examined in broth dilution method in order to determine their minimal inhibitory concentration (MICs). Microbial susceptibility testing was performed according to CLSI guidelines, respectively (17-18).

It was found that the tested compounds were not active against all tested microorganisms, except for the compound (**2d**). This compound affected the growth of *Bacillus subtilis, Staphyloccocus aureus* ATCC 25923, *Staphyloccocus aureus* ATCC 29213, *Staphyloccocus aureus* NCTC 4163, *Staphyloccocus aureus* ATCC 6538P and *Enterococcus hirae* ATCC 10541.

The results of the polycratic analysis display regular dependence between retention values and the concentration of the organic modifier (Figures 1, 2). The R_M values decrease linearly with an increase of the concentration of the organic solvent, which is in accordance with Soczewiński-Wachtmeister equation describing dependence of retention on the volume fraction of an organic modifier in the aqueous mobile phase (20):

$$R_M = R_{MW} + S\phi,$$

where ϕ is the volume fraction of organic solvent in an aqueous-organic solvent mixture, *S* is the slope of

Table 3. Linear logP_G and lipophilicity parameters dependences described by the equation of: log P_G = ax + b

	x	a	b	R^2	n	s	F
methanol-water	ϕ_o	13,5872	-5,1442	0,5315	20	0,6413	20,42
	S	-0,925	0,713	0,4925	20	0,6675	17,47
	$\mathbf{R_{Mw}}$	1,158	1,2425	0,5121	20	0,6545	18,89
acetonitrile-water	ϕ_o	20,193	-8,3422	0,633	20	0,5676	31,05
	S	-1,3992	0,6606	0,6842	20	0,5266	38,99
	$\mathbf{R_{Mw}}$	1,9314	0,0918	0,7111	20	0,5037	44,29

the regression curve and, R_{MW} (lipophilicity index) is the retention parameter for pure water as the eluent.

Determination of the retardation factors of the examined substances in RP-TLC in polycratic conditions allows, thanks to the already mentioned Soczewiński-Wachtmeister equation, to determine chromatographic parameters of lipophilicity: R_{Mw}, S and ϕ_0.

Chromatographic parameters of lipophilicity (R_{Mw}, *S and* ϕ_0) determined by RP-TLC in the analyzed elution systems (Table 2) were mutually correlated also using the $\log P_G$ calculated by Ghose et al. atomic algorithm by the use of QSAR properties module using HyperChem 5.1. software.

Figure 3 illustrates correlation between R_{Mw} values obtained by the use of different mobile phases. According to Biagi (21), the R_{Mw} values, as the retention parameters of substances in pure water should not depend on the type of an organic modifier used:

$$R_{Mw(ACN)} = 0.6208(\pm 0.0795)\, R_{Mw(MeOH)} + 0.5527(\pm 0.1679)$$

n = 20; R = 0.8786; s = 0.1954; F = 60.92

These observations are confirmed by the quite high correlation of R_{Mw} parameters from both elution systems used.

$$\phi_{0(ACN)} = 0.6909(\pm 0.0586)\, \phi_{0(MeOH)} + 0.1468(\pm 0.0378)$$

n = 20; R = 0.9409; s = 0.0125; F = 138.89

This is in accordance with Walko et al. (22). Taking into account a very high correlation coefficient and the fact that the slope of the regression curve is close to 1, both lipophilicity ϕ_0 scales may be considered as the same.

Figures 5 and 6 illustrate the correlations between the values of two chromatographic parameters of lipophilicity R_{Mw} and S obtained in the analyzed elution systems.

Both lipophilic scales display very high intercorrelation independently of the kind of the organic modifier used, which is confirmed by the correlation coefficient values presented below:

$$S_{(MeOH)} = -1.2215(\pm 0.0292)\, R_{Mw(MeOH)} - 0.6345(\pm 0.0616)$$

n = 20; R = 0.9949; s = 0.0717; F = 1750.52

and

$$S_{(ACN)} = -1.3434(\pm 0.0399)\, R_{Mw(ACN)} - 0.605(\pm 0.0741)$$

n = 20; R = 0.9922; s = 0.0692; F = 1134.07

As the relation of S to R_{Mw} is a constant value when speaking of strictly related substances, the so called "slope analysis" is used for determination of the relationship degree of the congeners. This dependence is disturbed, when the analyzed substances differ as for the donor-acceptor properties of the hydrogen bonds (23).

We may, therefore, assume that the molecular mechanism of retention is similar in case of all the analyzed compounds, which probably is connected with their similar structure and physico-chemical properties.

As retention mechanisms in liquid chromatography, in the system of the reversed phases are similar to the mechanisms of passive infiltration through the membranes of biologically living organisms, one should expect that biological activity of the studied derivatives should be similar, too.

Log P_G parameter being a universal reference system was correlated with chromatographic lipophilicity parameters obtained by TLC technique.

These correlations seem to be quite low, which, to a certain degree, may be explained by the imperfection of the calculation method applied. Computer programs basing on the fragmentation method of Ghose do not take into consideration, the differences in the placement of substituents and do not differentiate configuration isomers. One should assume, then that the achieved experimental lipophilicity parameters better reflect the real physico-chemical properties of the studied derivatives.

In order to understand why compound (**2e**), with 4-fluorophenyl group, is inactive while (**2d**), which possesses 2-fluorophenyl substituents, shows interesting activity against *Staphyloccus* spp., *Bacillus subtilis* and *Enterococcus hirae* it will be very interesting to evaluate antimicrobial activity of new 1-substituted-4-(2-fluorophenyl/4-fluorophenyl) thiosemicarbazides. Studies on the influence of 2-fluorophenyl and 4-fluorophenyl substituents in the position 4 of 1-substitued-thiosemicarbazide are in progress and it will be the subject of the next paper.

REFERENCES

1. Mekuskiene G., Gaidelis P., Vainilavicius P.: Pharmazie 53, 94 (1998).
2. Demirayak S., Benkli K., Güven K.: Eur. J. Med. Chem. 35, 1037 (2000).
3. Hui X-P., Zhang L-M., Zhang Z-Y., Wang Q., Wang F.: J. Chin. Chem. Soc. 47, 535 (2000).
4. Sahin G., Palaska E., Kelicen P., Demirdamar R., Altmok G.: Arzneimittelforschung 51, 478 (2001).
5. Holla B.S., Sarojini B.K., Rao B.S., Akberali P.M., Kumari N.S., Shetty V.: Farmaco 56, 565 (2001).

6. Eweiss N.F., Bahajaj A.A., Elsherbini E.A.: J. Heterocycl. Chem. 23, 1451 (1986).
7. Tozkoparan B., Kupeli E., Yesilada E., Isik S., Ozalp M., Ertan M.: Arzneimittelforschung 55, 533 (2005).
8. Shivarama Holla B., Sooryanarayana Rao B., Sarojini B.K., Akberali P.M., Suchetha Kumari N.: Eur. J. Med. Chem. 41, 657 (2006).
9. Al-Soud Y.A., Al-Dweri M.N., Al-Masoudi N.A.: Farmaco 59, 775 (2004).
10. Collin X., Sauleau A., Coulon J.: Bioorg. Med. Chem. Lett. 13, 2601 (2003).
11. Amir M., Khan M.S.Y., Zaman M.S.: Ind. J. Chem. 43B, 2189 (2004).
12. Palaska E., Sahin G., Kelicen P., Duirlu N.T., Altinok G.: Farmaco 57, 101 (2002).
13. Wujec M., Kosikowska U., Paneth P., Malm A.: Heterocycles 71, 2617 (2007).
14. Rollas S., Büyüktimkin S., Çevikbas A.: Arch. Pharm. 324, 189 (1991).
15. Hazzaa A.A.B., Labouta I.M., Kassem M.G.: Arch. Pharm. Chem. Sci. Ed. 11, 43 (1983).
16. Galabov A.S., Galabov B.S., Neykova N.A.: J. Med. Chem. 23, 1048 (1980).
17. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disc Susceptibility Tests; Approved Standard M2-A8. Clinical and Laboratory Standards Institute, Wayne, Pa. 2003.
18. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard M7-A6. Clinical and Laboratory Standards Institute, Wayne, Pa. 2003.
19. Pitucha M., Wujec M., Dobosz M.: J. Chin. Chem. Soc. 54, 69 (2007).
20. Soczewiński E.,Wachtmeister C.A.: J. Chromatogr. 7, 311 (1962).
21. Biagi G.L., Barbaro A.M., Sapone A., Recanatini M.: J. Chromatogr. 662, 341 (1994).
22. Valko K., Sleger P.: J. Chromatogr. 631, 46 (1993).
23. Biagi G.L., Barbaro A.M., Sapone A., Recanatini M.: J. Chromatogr. 669, 246 (1994).

Received: 12. 05. 2008