CROATICA CHEMICA ACTA
CCACAA, ISSN 0011-1643, e-ISSN 1334-417X
Croat. Chem. Acta **83** (3) (2010) 299–306.
CCA-3420

Original Scientific Article

Determination of the Lipophilicity of Some New Derivatives of Thiosemicarbazide and 1,2,4-triazoline-5-thione with Potential Antituberculosis Activity

Monika Pitucha,[a,*] Beata Polak,[b] Marta Swatko-Ossor,[c] Łukasz Popiołek,[a] and Grażyna Ginalska[c]

[a]*Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Lublin, Staszica 6, 20-081 Lublin, Poland*
[b]*Department of Physical Chemistry, Chair of Chemistry, Faculty of Pharmacy, Medical University of Lublin, Staszica 6, 20-081 Lublin, Poland*
[c]*Department of Biochemistry, Faculty of Pharmacy, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland*

RECEIVED APRIL 16, 2009, REVISED DECEMBER 9, 2009, ACCEPTED MARCH 10, 2010

Abstract. The chromatographic behavior of newly obtained derivatives of thiosemicarbazide and 1,2,4-triazoline-5-thione was determined. The lipophilicity was confirmed by the use of the Reversed Phase Thin-Layer Chromatography (RP-TLC) method. For both groups of solutes the lipophilicity depended on the substituents. All obtained compounds were tested for their antimycotic activity. The strongest antituberculosis activity was observed for 4-(2-iodophenyl)-1-(pyridine-4-ylacetyl)thiosemicarbazide **4** and 4-phenyl-3-(pyridine-4-ylmethyl)-1,2,4-triazoline-5-thione **27**.

Keywords: thiosemicarbazide, 1,2,4-triazoline-5-thione, *Mycobacterium tuberculosis*, lipophilicity

INTRODUCTION

Today, tuberculosis, right after the HIV infection,[1] is a worldwide health threat. 30 million new cases are reported each year. Statistics show that approximately 3 million people will die annually from tuberculosis.[2] For this reason, research into new drugs is carried out. It was noticed that compounds with an azole group may be regarded as an effective antitubercular class of drugs. According to reports, they inhibit bacteria by blocking the biosynthesis of certain bacterial lipids and/or by additional mechanisms.[3,4] Triazole, in particular, substituted the 1,2,4-triazoles and the open-chain thiosemicarbazide counterparts of the 1,2,4-triazole are among the various heterocycles that have received the most attention during the last two decades as potential antimicrobial agents.[5–7] It is known that the hydrophobic character of substances has a major effect on biological activity.[8] The lipophilicity of the compounds plays an important role in the penetration of solutes into the bacterial cell tissue.[9]

The lipophilicity character of compounds may be defined in various ways. The one most frequently applied is a partition coefficient, *P*, or its decimal logarithm, log *P*, which represents the tendency of a molecule to partition itself between the organic and aqueous phases. The traditional "shake-flask" partition method between *n*-octanol and water is often substituted by chromatographic approaches (Reversed-Phase High Pressure Liquid Chromatography (RP-HPLC) or RP-TLC methods).[10–12] It was determined for various groups of derivatives that thanks to using the chromatographic technique it could be possible to explain differences between the biological activity of compounds with a similar structure.[13–17] This investigation could also suggest the strategy for the future synthesis of more active solutes.

The aim of the study reported here was broad research into two series of newly synthesized derivatives of thiosemicarbazide and 1,2,4-triazol-5-thione. These investigations include the determination of lipophilicity by the chromatographic method, as well as the antituberculosis activity of solutes.

EXPERIMENTAL SECTION

All chemicals were purchased from Merck Co. or Lancaster (Gdańsk, Poland) and used without further purification. Melting points were determined in a Fisher-Johns block and were not corrected. Elemental analysis was made on a Perkin-Elmer 2400 CHN Analyzer and the data were within ± 0.4 % of the theoretical values. The ^{1}H NMR spectra were recorded on a Bruker AC

* Author to whom correspondence should be addressed. (E-mail: monika.pitucha@am.lublin.pl)

200F instrument (300 MHz) in DMSO-d_6 with TMS as an internal standard. The IR spectra were recorded in KBr on a Perkin-Elmer 1725X FTIR spectrometer. The purity of obtained compounds was checked by Thin-Layer Chromatography TLC on aluminium oxide 60 F_{254} plates. The solvent volume ratio in TLC mobile phase used for checking the purity of purchased compounds was $\psi(CHCl_3,C_2H_5OH)$ = 10:1 and 10:2. Detection was conducted with UV light or iodine vapor.

Some compounds (**4-9**, **11**, **12, 14, 16, 18-27**) were prepared according to the procedure reported earlier.[18–26]

Synthesis of thiosemicarbazides (**1-8**)
A mixture of pyridine-4-acetic acid hydrazide (1.65 g, 0.01 mol) and appropriate isothiocyanate (0.01 mol) was heated in an oil bath at 60–70 ºC for 15 h. The product was washed with diethyl ether to remove the unreacted isothiocyanate, filtered off and dried as well as crystallized from ethanol.

4-(4-Iodophenyl)-1-(pyridine-4-ylacetyl)-thiosemcarbazide (**1**)
Yield 2.2 g (78 %); m.p. 145–148 ºC; ^{1}H NMR (DMSO) δ/ppm: 3.58 (s, 2H, CH_2), 7.24–7.70 (m, 4H, CH_{arom}), 8.46–8.51 (m, 4H, CH_{arom}), 9.47 (s, H, NH), 9.72 (s, 1H, NH), 10.26 (s, 1H, NH). *Anal.* Calcd. for $C_{14}H_{13}IN_4OS$ (M_r = 285.34): C 58.92, H 4.59, N 19.63 %; found: C 59.14, H 4.82, N 19.21 %.

4-(4-Nitrophenyl)-1-(pyridine-4-ylacetyl)-thiosemcarbazide (**2**)
Yield 2.3 g (69 %); m.p. 185–187 ºC; ^{1}H NMR (DMSO) δ/ppm: 3.61 (s, 2H, CH_2), 7.26–7.92 (m, 4H, CH_{arom}), 8.21–8.52 (m, 4H, CH_{arom}), 9.57 (s, 1H, NH), 10.05 (s, 1H, NH), 10.37 (s, 1H, NH). *Anal.* Calcd. for $C_{14}H_{13}N_5O_3S$ (M_r = 331.35): C 50.74, H 3.95, N 21.13 %; found: C 51.01, H 4.02, N 21.31 %.

4-(4-Chlorophenyl)-1-(pyridine-4-ylacetyl)-thiosemcarbazide (**3**)
Yield 2.3 g (73 %); m.p. 80–82 ºC; ^{1}H NMR (DMSO) δ/ppm: 3.58 (s, 2H, CH_2), 7.24–7.61 (m, 4H, CH_{arom}), 8.46–8.51 (m, 4H, CH_{arom}), 9.72 (s, 1H, NH), 9.77 (s, 1H, NH), 10.26 (s, 1H, NH). *Anal.* Calcd. for $C_{14}H_{13}ClN_4OS$ (M_r = 320.79): C 52.41, H 4.08, N 17.46 %; found: C 52.71, H 4.32, N 17.79 %.

4-(2-Fluorophenyl)-1-(pyridine-4-ylacetyl)-thiosemcarbazide (**4**)[18]
Yield 2.07 g (68 %); m.p. 148–149 ºC.

4-[2-(4-Morpholinoethyl)]-1-(pyridine-4-ylacetyl)-thiosemicarbazide (**5**)[18]
Yield 2.68 g (83 %); m.p. 170–172 ºC.

1-[(1-Methylpyrrol-2-yl)acetyl]-4-phenylthiosemicarbazide (**6**)[19]
Yield 2.36 g (82 %); m.p. 173–175 ºC.

4-Ethyl-1-(pyridine-3-ylcarbonyl)-thiosemicarbazide (**7**)[20]
Yield 1.91 g (85 %); m.p. 176–178 ºC.

4-Allyl-1-(pyridine-3-ylcarbonyl)-thiosemicarbazide (**8**)[21]
Yield 1.70 g (72 %); m.p. 190–191 ºC.

Synthesis of 3,4-disubstituted-1,2,4-triazoline-5-thiones (**9-12**)
A mixture of respective thiosemicarbazides (0.01 mol) and 2 % aqueous solution of sodium hydroxide (30 ml) was refluxed for 2h. The product of this reaction was precipitated by the addition of 3 mol L^{-1} dilute hydrochloric acid, filtered, dried and recrystallized from ethanol.

4-Ethyl-3-(pyridine-4-ylmethyl)-1,2,4-triazoline-5-thione (**9**)[18]
Yield 3.02 g (73 %); m.p. 204−206 ºC.

4-(4-Nitrophenyl)-3-(pyridine-4-ylmethyl)-1,2,4-triazoline-5-thione (**10**)
Yield 1.9 g (63 %); m.p. 225−28 ºC: ^{1}H NMR (DMSO) δ/ppm: 3.98 (s, 2H, CH_2), 7.07−7.71 (m, 4H, CH_{arom}), 8.31–8.41 (m, 4H, CH_{arom}), 13.99 (s, 1H, NH). *Anal.* Calcd. for $C_{14}H_{11}N_5O_2S$ (M_r = 313.33): C 53.66, H 3.53, N 22.35 %; found: C 53.21, H 3.82, N 22.21%.

4-(2-Fluorophenyl)-3-(pyridine-4-ylmethyl)-1,2,4-triazoline-5-thione (**11**)[18]
Yield 2.06 g (75 %); m.p. 273–275 ºC.

4-Allyl-3-(pyridine-4-ylmethyl)-1,2,4-triazoline-5-thione (**12**)[18]
Yield 1.81 g (78 %); m.p. 210–211 ºC.

4-Phenyl-3-(1,2,4-triazol-1-ylmethyl)-1,2,4-triazoline-5-thione (**14**)[22]
Yield 1.83 g (71 %); m.p. 256–258 ºC.

4-(4-Fluorophenyl)-3-(pyridine-4-ylmethyl)-1,2,4-triazoline-5-thione (**15**)
Yield 1.9 g (71 %); m.p. 225−228 ºC; ^{1}H NMR (DMSO) δ/ppm: 3.90 (s, 2H, CH_2), 7.30–8.39 (m, 8H, CH_{arom}), 13.99 (s, 1H, NH). *Anal.* Calcd. for $C_{14}H_{11}FN_4S$ (M_r = 267.33): C 62.90, H 4.14, N 20.96 %; found: C 62.66, H 4.62, N 20.11 %.

4-(4-Methoxyphenyl)-3-(pyridine-4-ylmethyl)-1,2,4-triazoline-5-thione (**16**)[18]
Yield 3.92 g (76 %); m.p. 226–228 ºC.

3-[(1-Methylpyrrol-2yl)methyl]-4-(4-methoxyphenyl)-1,2,4-triazoline-5-thione (**18**)[19]
Yield 2.10 g (70 %); m.p. 144–145 ºC.

4-Benzyl-3-[(1-methylpyrrol-2yl)methyl]-1,2,4-triazoline-5-thione (**19**)[19]
Yield 2.16 g (76 %); m.p. 119–121 ºC.

3-Methyl-4-phenyl-1,2,4-triazoline-5-thione (**20**)[23]
Yield 1.49 g (78 %); m.p. 220–221 ºC.

3-[(1-Methylpyrrol-2yl)methyl]-4-phenyl-1,2,4-triazoline-5-thione (**21**)[19]
Yield 2.05 g (76 %); m.p. 183–185 ºC.

4-Ethyl-3-[(1-methylpyrrol-2yl)methyl]-1,2,4-triazoline-5-thione (**22**)[19]
Yield 1.66 g (75 %); m.p. 174–176 ºC.

4-Bromophenyl-3-(pyridine-4-ylmethyl)-1,2,4-triazoline-5-thione (**23**)[19]
Yield 2.34 g (74 %); m.p. 252–254 ºC.

4-Phenyl-3-(pyridine-3-yl)-1,2,4-triazoline-5-thione (**24**)[20]
Yield 1.83 g (72 %); m.p. 276–278 ºC.

4-Allyl-3-(pyridine-3-yl)-1,2,4-triazoline-5-thione (**25**)[24]
Yield 1.42 g (70 %); m.p. 170–173 ºC.

4-Phenyl-3-(pyridine-4-ylmethyl)-1,2,4-triazoline-5-thione (**27**)[18]
Yield 2.01 g (75 %); m.p. 128–129 ºC.

Synthesis 1,3,4-trisubstituted-1,2,4-triazoline-5-thione (13, 17, 26)

1-Allyl-4-phenyl-3-(pyridine-3-yl)-1,2,4-triazoline-5-thione (**13**)
0.23 g (0.01 gram-atom) of sodium was added to 5 ml of anhydrous ethanol, placed in a three-neck flask equipped with a reflux condenser closed with a tube of $CaCl_2$. The content was mixed till sodium dissolved completely and then 0.01 mole 4-phenyl-3(pyridin-3-yl)-1,2,4-triazoline-5-thione was added. Then, 0.01 mole of allyl bromide was added drop by drop. The content of the flask was mixed for 4 h and left at room temperature for 12 h. Then, 10 ml of anhydrous ethanol was added and heated for 1 h. It was filtered off inorganic compounds. After cooling, the precipitate was filtered and crystallized from ethanol.

Yield 1.9 g (67 %); m.p. 148–150 ºC; IR (KBr) ν_{max}/cm^{-1}: 3054 (CH_{arom}), 2943, 1411 (CH_{aliph}), 1636 (C=N), 1455 (C−N); ^{1}H NMR (DMSO) δ/ppm: 5.10–5.14 (m, 2H, CH_2), 5.23–5.32 (m, 2H, CH_2), 5.89–6.02 (m, 1H, CH), 7.37–8.58 (m, 9H, CH_{arom}). *Anal.* Calcd. for $C_{16}H_{14}N_4S$ (M_r=294.37): C 65.28, H 4.79, N 19.03 %; found: C 65.77, H 4.82, N 18.88%.

1-(2-Chloroethyl)-4-ethyl-3-(pyridine-3-yl)-1,2,4-triazoline-5-thione (**17**)
2.5 g (0.01 mole) of 1-(2-hydroxyethyl)-4-ethyl-3-(pyridin-3-yl)-1,2,4-triazoline-5-thione[26] was dissolved in 25 ml of dry chloroform cooled (−5 ºC) and stirred. Then 4.8 g (0.04 mole) of freshly distilled thionyl chloride was added drop by drop. This content was mixed for 2 h. Later the reaction mixture was heated to moderate boiling for 4 h. The solvent and the excess of $SOCl_2$ were removed by distillation under reduced pressure. Dry residue was then cooled and the product was crystallized from anhydrous ethanol.
Yield 1.7 g (63 %); m.p. 168–170 ºC; ^{1}H NMR (DMSO) δ/ppm: 1.27 (t, 3H, CH_3), 3.67 (t, 2H, CH_2), 3.97 (t, 2H, CH_2), 4.07 (t, 2H, CH_2), 8.17–9.20 (m, 4H, CH_{arom}). *Anal.* Calcd. for $C_{11}H_{13Cl}N_4S$ (M_r=268.76): C 49.15, H 4.87, N 20.84 %; found: C 51.18, H 4.93, N 20.44%.

4-Ethyl-1-hydroxymethyl-3-(pyridine-3-yl)-1,2,4-triazoline-5-thione (**26**)[26]
Yield 2.05 g (87 %); m.p. 145–146 ºC.

Thin-layer chromatographic analysis

Thin-layer chromatography was performed on 10 × 10 cm TLC plates coated with RP-18 F_{245}. Methanol-water mixtures were used as mobile phases. The methanol volume fraction was varied from 60 to 90 % in 5 % steps. Plates were developed to a distance of 9 cm at room temperature in a horizontal DS chambers (Chromes, Lublin, Poland) and after drying visualized under λ = 254 nm UV light. Each experiment was performed four time.

Antimycobacterial activity

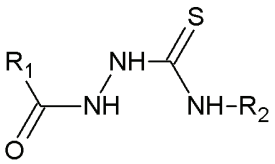
Antituberculosis activities of the synthesized compounds were screened *in vitro* against mycobacterium strains: *Mycobacterium smegmatis*, *Mycobacterium phlei* and avirulent strain *Mycobacterium H$_{37}$Ra* using the broth dilution method for determination of the Minimum Inhibitory Concentration (MIC) to inhibit the growth of microorganisms. MIC was defined as the highest dilution of compound required to give total inhibition of bacterial growth judged by the lack of turbidity in the tube.[27]

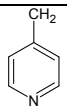
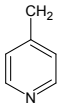
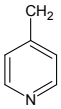
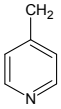
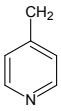
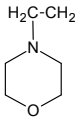
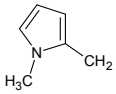
Inocula for susceptibility testing were from a suspension of microorganisms isolated earlier on Lowenstein medium, equivalent to a McFarland No. 1 standard. Turbidity of the suspension was measured using nefelometer BD PhoenixSpec, Becton, Dickinson and Company USA. Then the culture was added to each vial containing the test drug. A control was prepared in the same manner as a drug containing vial. The vials with compounds were incubated 72 h at 37 ºC and the control was stored at 4 ºC. As a standard drug the Ryfampicin and Streptomycin were used for comparison.

RESULTS AND DISCUSSION

The thiosemicarbazide derivatives (**1-8**) were obtained in the reaction of an appropriate hydrazide carboxylic acid with isothiocyanates. The cyclization reaction of these compounds led to 3,4-disubstituted 1,2,4-triazoline-5-thione (**9-12**, **14-16**, **18-25**, **27**). In ^{1}H NMR spectra of triazole derivatives the proton signals for the

Table 1. Chemical structure of thiosemicarbazide derivatives used in this study



Comp.	Substituents	
	R_1	**R_2**
1		4-IC_6H_4
2		4-$NO_2C_6H_4$
3		4-ClC_6H_4
4		2-FC_6H_4
5		
6		C_6H_5
7	3-NC_5H_4	C_2H_5
8	3-NC_5H_4	$CH_2CH{=}CH_2$

group −NH−C=S were observed at 13.99 ppm. Some of cyclized products were subjected to a reaction with allyl bromide (**13**), formaldehyde (**26**), as well as to chloroethylation (**17**). The signal proton characteristic for the −NH−C=S group, found in the 3,4-disubstituted 1,2,4-triazoline-5-thiones, was not observed in the ^{1}H NMR spectra of 1,3,4-trisubstituted triazoles. The reactions were performed according to Scheme 1. Structures of all derivatives used in this study are presented in Table 1 and Table 2.

The lipophilicity of chosen solutes was chromatographically determined.

The values of the retardation factor R_F (the ratio of the migration distance of the analyte to the migration distance of the mobile phase) were used to calculate R_M values according to Bate-Smith and Westall equation[28]

$$R_M = \log\left(\frac{1}{R_F} - 1\right) \quad (1)$$

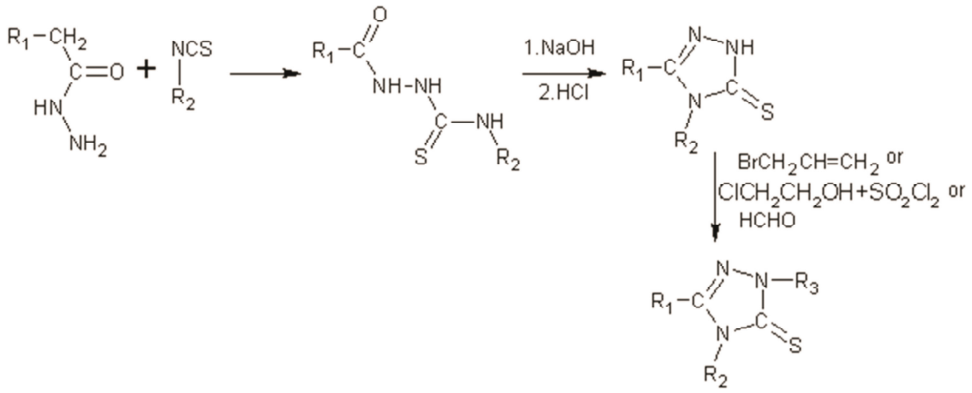
Relationships R_M values *vs.* methanol volume fraction in the mobile phase allowed to determine the R_{M0} and φ values according to the Soczewinski-Wachtmeister equation[29]

$$R_M = R_{M0} + bC \quad (2)$$

where C is the methanol volume fraction in the mobile phase; R_{M0} is the R_M value obtained for pure water, φ was the ratio between b and R_{M0}.

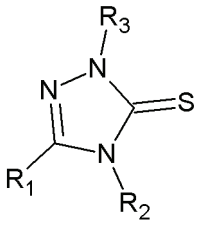
The relative lipophilicity, expressed as R_{M0} values and the parameters obtained from the regression analysis, such as the slopes (a), the correlation coefficient square (R^2), the standard deviation of estimation (SD) and the values of F-test of significance (F), were listed in Table 3. The lipophilicity determined was in the range of 3.29–0.65. Comparison of the relative lipophilicity of a solute series revealed that higher values were obtained for the 1,2,4-triazoline-5-thione derivatives. For both groups of solutes the relative lipophilicity depended on the substituents (R_1, R_2, R_3).

The substituent in the (R_2) position of thiosemicarbazide derivatives strongly influenced relative lipo-



Scheme 1.

Table 2. Chemical structure of 1,2,4-triazol-5-thiones used in this study



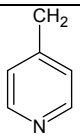
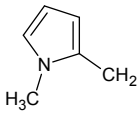
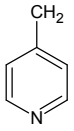
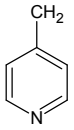
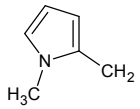
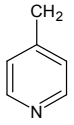
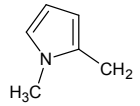
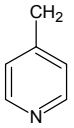
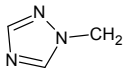
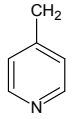
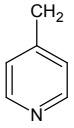
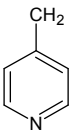
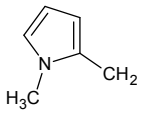
	Substituents				Substituents		
Comp.	**R_1**	**R_2**	**R_3**	Comp.	**R_1**	**R_2**	**R_3**
9		C_2H_5	H	**19**		$CH_2C_6H_5$	H
10		4-$NO_2C_6H_5$	H	**20**	CH_3	C_6H_5	H
11		2-FC_6H_4	H	**21**		C_6H_5	H
12		$CH_2CH{=}CH_2$	H	**22**		C_2H_5	H
13	3-NC_5H_4	C_6H_5	$CH_2CH{=}CH_2$	**23**		4-BrC_6H_4	H
14		C_6H_5	H	**24**	3-NC_5H_4	C_6H_5	H
15		4-FC_6H_4	H	**25**	3-NC_5H_4	$CH_2CH{=}CH_2$	H
16		4-$CH_3OC_6H_4$	H	**26**	3-NC_5H_4	C_2H_5	CH_2OH
17	3-NC_5H_4	C_2H_5	CH_2CH_2Cl	**27**		C_6H_5	H
18		4-$CH_3OC_6H_4$	H				

Table 3. Parameters of the linear correlations between the R_M values of the solutes of investigated compounds and the volume fraction of methanol in the mobile phase and the statistic parameters (R^2 – correlation coefficient square; *F*-test of significance, $n = 7$; SD-standard deviation of estimation)

No	$-a$	R^2	R_{M0}	$-\varphi$	*F*	SD
1	0.026±0.008	0.9261	2.104±0.008	0.012	62.64	0.0868
2	0.016±0.006	0.9146	1.168±0.417	0.013	53.57	0.0567
3	0.019±0.004	0.9682	1.420±0.302	0.013	152.40	0.0411
4	0.012±0.002	0.9692	0.645±0.182	0.018	157.24	0.0248
5	0.010±0.003	0.9296	1.035±0.244	0.010	66	0.0332
6	0.020±0.003	0.9856	1.176±0,214	0.017	341.57	0.0291
7	0.017±0.002	0.9878	0.668±0.165	0.026	404.82	0.0225
8	0.020±0.004	0.9742	0.927±0.280	0.021	189.06	0.0381
9	0.017±0.003	0.9833	0.970±0.195	0.018	294.56	0.0265
10	0.023±0.003	0.9881	1.485±0.219	0.015	416.08	0.0298
11	0.017±0.005	0.9369	1.024±0.394	0.017	74.21	0.0537
12	0.017±0.004	0.9646	1.061±0.287	0.016	136.36	0.0390
13	0.030±0.007	0.9561	2.444±0.562	0.012	108.82	0.0765
14	0.018±0.005	0.9497	0.803±0.362	0.023	94.39	0.0492
15	0.022±0.006	0.9541	1.379±0.424	0.016	103.86	0.0577
16	0.024±0.004	0.9767	1.540±0.316	0.015	209.21	0.0430
17	0.018±0.004	0.9636	1.448±0.297	0.012	132.30	0.0404
18	0.034±0.008	0.9575	2.586±0.618	0.013	112.69	0.0841
19	0.029±0.006	0.9657	2.039±0.469	0.014	140.66	0.0638
20	0.024±0.009	0.9019	1.393±0.687	0.017	45.95	0.0935
21	0.042±0.010	0.9568	3.293±0.770	0.013	110.61	0.1047
22	0.021±0.004	0.9730	1.354±0.310	0.016	180.14	0.0422
23	0.031±0.006	0.9754	2.235±0.424	0.014	198.09	0.0577
24	0.025±0.002	0.9940	2.356±0.171	0.011	823.15	0.0232
25	0.019±0.005	0.9521	1.155±0.370	0.016	99.32	0.0503
26	0.016±0.006	0.9114	0.943±0.437	0.017	51.43	0.0595
27	0.019±0.003	0.9817	1.112±0.222	0.017	268.11	0.0303

philicity. The decrease of the R_{M0} value was in the following order: 4-iodophenyl (**1**) > 4-chlorophenyl(**3**) > phenyl (**6**) > 4-nitrophenyl (**2**) > morpholinoethyl (**5**) > allyl (**8**) > ethyl (**7**) > 2-fluorophenyl (**4**).

The second group, 1,2,4-triazoline-5-thione derivatives, were divided into several subgroups. The first one was bearing pyridine-4-ylmethyl as R_1. The relative lipophilicity of these compounds decreased in the following order: 4-methoxyphenyl (**16**) > 4-nitrophenyl (**10**) > 4-fluorophenyl (**15**) > phenyl (**27**) > allyl (**12**) > 2-fluorophenyl (**11**) > ethyl (**9**).

The second group possesses pyridine-3-yl as R_1. The relative lipophilicity of these compounds decreased in the following order: phenyl (**24**) > 4-bromophenyl (**23**) > ethyl and chloroethyl (**17**) > allyl (**25**) > ethyl and hydroxymethyl (**26**).

The third group possesses 1-methylpyrrol-2-ylmethyl as R_1. The relative lipophilicity in this group decreased in the following order: phenyl (**21**) > 4-methoxyphenyl (**18**) > benzyl (**19**)> ethyl (**22**).

The fourth group has a phenyl substituent as R_2. The relative lipophilicity decreased in the order of pyridine-3-yl and allyl (**13**) > pyridine-3-yl (**24**) > methyl (**20**) > 1,2,4-triazol-1ylmethyl (**14**).

The relative lipophilicity of solutes bearing the halogens changed with the increase of molecular mass of halogen. Among the thiosemicarbazides the highest lipophilicity was observed for a solute (**1**) with iodine,

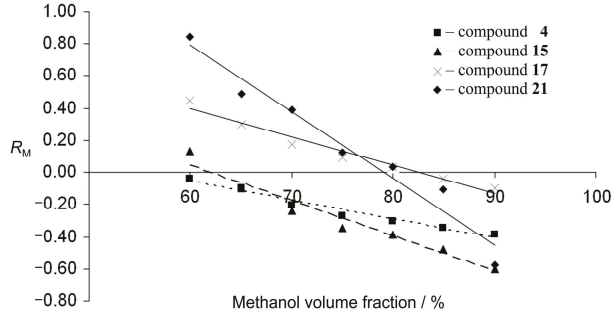


Figure 1. The examples of correlations between the R_M values and methanol volume fraction in the mobile phase for solutes **4**, **15**, **17**, **21**.

the smallest for fluorine (**4**). Among the 1,2,4-triazoline-5-thiones the highest lipophilicity was observed for a solute possessing bromine (**23**), the smallest for fluorine (**11**). Also the position of fluorine in the phenyl ring influence R_{M0} values. The highest was observed when fluorine was in position 4 (compare solutes **15** and **11**). The examples of the correlation between the R_M values and the methanol volume fraction in the mobile phase for solutes **4**, **15**, **17**, **21** was presented in Figure 1.

The higher R_{M0} values indicate greater lipophilicity. Arranging the solutes in the order of decreasing lipophilicity resulted in the following list:

21>18>13>24>23>1>19>16>17>10>3>20>22>15 >6>2>25>27>12>5>11>9> 26>8>14>7>4

The *in vitro* antituberculosis activities of all compounds were evaluated against mycobacterium strains: *Mycobacterium smegmatis*, *Mycobacterium phlei* and *Mycobacteium $H_{37}Ra$* using the broth dilution method. According to our results, some of them showed promising antituberculosis activity against *Mycobacterium phlei* (Table 4). The most effective derivatives possess a 2-fluorophenyl substituent of 1-(pyridine-4-ylacetyl)-thiosemcarbazide (**4**) and a phenyl group of 3-(pyridine-4-ylmethyl)-1,2,4-triazoline-5-thione (**27**).

Table 4. Antituberculosis activities of obtained compounds

Minimum inhibitory concentration /μg ml^{-1}			
Comp.	*M. smegmatis*	*M. Phlei*	*M. $H_{37}Ra$*
4	R[(a)]	128	256
10	R[(a)]	256	R[(a)]
26	256	256	512
27	256	128	R[(a)]
Streptomycin	64	128	32
Rifampicin	64	32	32

[(a)] >1000 μg = R (resistant)

CONCLUSION

The lipohilicity of newly obtained derivatives of thiosemicarbazides and 1,2,4-triazoline-5-thiones were determined by thin-layer chromatography method. The smallest values were determined for 3-[(1-methylpyrrol-2-yl)methyl]-4-phenyl-1,2,4-triazoline-5-thione (**21**). The highest values of lipophilicity were determined for 4-(2-fluorophenyl)-1-(pyridine-4-ylacetyl)-thiosemcarbazide (**4**).

The biological activity of newly synthesized compounds against *Mycobacterium sp.* was investigated. The highest antituberculosis activity was observed against *Mycobacterium pheli.* The minimum inhibitory concentration of the solute (**4**) and (**27**) was similar to Streptomycin (standard). The results obtained will be used to further study the biological activity obtained compounds.

REFERENCES

1. P. Nunn, B. Williams, K. Foyd, C. Dye, G. Elzinga, and M. Raviglione, *Nat. Rev. Immunol.* **5**, (2005) 819–826.
2. D. E. Snider, M. Raviglione, and A. Kochi, *Global burden of tuberculosis. In: Bloom, B.* (Ed.), Tuberculosis: Pathogenesis, Protection and Control. ASM Press, Washington, DC, 1994, 3–11.
3. K. Babaoghe, M. A. Page, V. C. Johns, J. H. Naismith, and R. E. Lee Novel, *Bioorg. Med. Chem. Lett.* **13** (2003) 3227–3229.
4. M. R. Shiradkar, S. V. Bhandari, R. P. Kale, A. Laghate, and A. Rathi, *Asian J. Chem.* **18** (2006) 2700–2704.
5. M. B. Gravestock and M. Barry, EU Patent EP 94,146 B1, 1984.
6. B. Hirsch, D. Lohmann, G. Menzel, G. Schuster, and E. Stenz, German Democratic Republic Patent DD 2,34,003, 1983.
7. T. Ikeda and K. Tada, EU Patent EP 2,62,589 B1, 1988.
8. V. Pliška, B. Testa, and H. Van de Waterbeemd, *Lipophilicity in Drug Action and Toxycology,* WCH, Weinheim 1996.
9. K. Novak., B. Noszal, I. Hermecz, G. Kereszturi, B. Podanyi, and G. Szasz, *J. Pharm. Sci.* **79** (1990) 1023–1028.
10. K. Więckowski, A. Czaja, A. Wozniak, A. Musiał, and B. Malawska, *J. Planar Chromatogr.* **20** (2007) 101–106.
11. M. Waksmundzka-Hajnos, D. Matosiuk, A. Petruczynik, and U. Kijowska-Murak, *Acta Chromatographica* **20** (2008) 563–573.
12. B. Malawska, K. Kulig, A. Bucki, P. Zbęk, and A. Więckowska, *Biomed. Chromatorg.* **22** (2008) 688–694.
13. J. Obińska and K. Kamiński, *Biomed. Chromatogr.* **20** (2006) 1185–1195.
14. D. Łażewska, P. Maludziński, E. Szymańska, and K. Kieć-Kononowicz, *Biomed. Chromatogr.* **21** (2007) 291–298.
15. M. Bajda, S. Boryczka, J. Wietrzyk, and B. Malawska, *Biomed. Chromatogr.* **21** (2007) 123–131.
16. A. Hawrył, D. Cichocki, and M. Waksmundzka-Hajnos, *J. Planar Chromatogr.* **21** (2008) 343–348.
17. A. Gumieniczek, A. Berecka, D. Matosiuk, and H. Hopkała, *J. Planar Chromatogr 20*, (2007) 261–265.
18. M. Pitucha, M. Wujec, and M. Dobosz, *Journal of Chinese Chemical Society* **54** (2007) 69–73.
19. M. Pitucha, M. Wujec, and M. Dobosz, *Annales UMCS, Sectio AA* **LIX** (2004) 144–153.
20. M. Dobosz., M. Pitucha, and M. Wujec, *Acta Polon. Pharm.* **53** (1996) 31–38.

21. Z. Ming-gen, S. Yu-fang, Z. Jiang-yu, L. Hai-bin, and Z. Ting-ting, *Shanxi Daxue Xuebao* **30** (2007) 64–67.
22. M. Dobosz and M. Sikorska, *Acta Polon. Pharm.* **51** (1994) 369–376.
23. M. Pitucha, M. Wujec, and M. Dobosz, *Annals of Polish Chemical Society* **3** (2004) 64–67.
24. N. A. Abdou, L. N. Soliman, and A. H. Abou Sier, *Bulletin of the Faculty of Pharmacy* **28** (1990) 29–31.
25. R. Iqbal, N. H. Rama, U. Yunus, A. Saeed, and K. Zamani, *Journal of the Chemical Society of Pakistan* **19** (1997) 145–149.
26. M. Wujec, M. Pitucha, and M. Dobosz, *Acta Polon. Pharm.* **61** (2004) 13–15.
27. D. B. Yuong, *Strategies for new drug development. In Tuberculosis; Bloom, B.R.* (Ed.). ASM Press, Washington, DC, 1994, 559–567.
28. E. C. Bate-Smith and R. G. Westall, *Biochim. Biophys. Acta* **4** (1950), 427–440.
29. E. Soczewiński and C. A. Wachtmeister, *J. Chromatogr.* **7** (1962) 311–320.

SAŽETAK

Određivanje lipofilnosti nekih novih derivata tiosemikarbazida i 1,2,4-triazolin-5-tiona s potencijalnom antituberkuloznom aktivnosti

Monika Pitucha,[a] Beata Polak,[b] Marta Swatko-Ossor,[c] Łukasz Popiołek[a] i Grażyna Ginalska[c]

[a]*Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Lublin, Staszica 6, 20-081 Lublin, Poland*
[b]*Department of Physical Chemistry, Chair of Chemistry, Faculty of Pharmacy, Medical University of Lublin, Staszica 6, 20-081 Lublin, Poland*
[c]*Department of Biochemistry, Faculty of Pharmacy, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland*

Određena su kromatografska svojstva novo dobivenih derivata tiosemikarbazida i 1,2,4-triazolin-5-tiona. Lipofilnost je potvrđena koristeći metodu reverzno-fazne tankoslojne kromatografije (engl. *Reversed Phase Thin-Layer Chromatography* (RP-TLC)). Za obje skupine otopljenih tvari lipofilnost je ovisila o supstituentima. Svi dobiveni spojevi su bili testirani za antimikotsku aktivnost. Najjača antituberkulozna aktivnost je uočena za 4-(2-jodofenil)-1-(piridin-4-ilacetil)tiosemikarbazid **4** i 4-fenil-3-(piridin-4-ilmetil)-1,2,4-triazolin-5-tion **27**.