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Article

Chromatographic and Calculation Methods for Analysis of the Lipophilicity of Newly Synthesized Thiosemicarbazides and their Cyclic Analogues 1,2,4-Triazol-3-thiones

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This paper describes the evaluation of the lipophilicity of newly synthesized thiosemicarbazides and their cyclic analogues 1,2,4-triazol-3-thiones obtained using experimental and calculated methods. Previous studies have shown these compounds have antibacterial activity. The chromatographic behavior of analyzed compounds was studied by reversed phase high performance liquid chromatography (RP-HPLC) and reversed phase thin layer chromatography (RP-TLC). The aqueous mobile phases containing methanol were used in order to determine retention parameter (R_M) and capacity factors (log k) of analyzed compounds. The lipophilicity parameters were obtained by linear extrapolation and they were compared with the calculated log P obtained using several software packages. The results indicate that both experimental chromatographic methods yielded similar results, and these methods are appropriate for determining the lipophilicity of analyzed compounds. High values of correlation coefficients between the log P values calculated using known algorithms (milogP, ALOGPs, AClogP, AlogP, MLOGP, KOWWIN, XLOGP2, XLOGP3) and the experimental data were obtained. Eight standard solutes with known log P_{OW} were analyzed under the same conditions as the tested substances in order to determine the log P_{HPLC} and log P_{TLC} parameters. A good correlation was obtained between log k_w (or R_{MW}) and the slope. All tested compounds were in agreement with the rule of five claims by Lipiński. The calculated log P values were experimentally confirmed (log P_{HPLC} and log P_{TLC}).

Keywords: lipophilicity, RP-HPLC, RP-TLC, log P_{OW}

Introduction

Synthesis and confirmation of identity of studied thiosemicarbazide derivatives and their cyclic analogues 1,2,4-triazole-3-thiones were described early.[1] In the cited work, the antibacterial activity of several compounds is presented.

It is well known that the biological activity of some substances is related to their lipophilicity. This parameter determines the bioavailability of the chemical compound and it has been important, in prediction of crossing biological barriers of drug molecules and its interactions with receptors. Lipophilicity is a chemical affinity for the lipid and aqueous phases, and the measure is the ratio of the equilibrium concentration (activity) of the solute in the two-phase system consisting of two immiscible solvents non-polar and polar ones.[2-4] For a long time, the distribution coefficient between *n*-octanol and water was used as an experimental lipophilicity index of the compound. However, due to some limitations and technical barriers, the other methods for determination of the lipophilicity were applied. The chromatographic techniques have proved to be an important alternative method for determination of the lipophilicity. The chromatographic techniques such as reversed phase thin layer chromatography (RP-TLC),[5-10] reversed phase high performance liquid chromatography (RP-HPLC),[11-14] microemulsion electrokinetic chromatography,[15] immobilized artificial membrane (IAM) chromatography,[16-18] biopartitioning micellar chromatography (BMC),[16,19] immobilized liposome chromatography (ILC) are commonly used.[16]

The logarithm of *n*-octanol-water partition coefficient log P_{OW} is the most frequently used parameter for measuring of lipophilicity and it has been shown that this system is a good model for many biological processes.[20] This parameter is also used as one of the standard properties identified by Moreno *et al*.[20] and Lipiński *et al*.[21] in the "rule of five" for drug-likemolecules.

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The classical shake flask method for determining lipophilicity has many disadvantages, i.e., it is time consuming, the quantitative analysis must be used, the log P_{OW} value is limited to the range from 2 to 4.[22] Therefore, many chromatographic methods have successfully been used to determine the lipophilicity of potential drugs. Both HPLC and TLC methods are easy, fast, reproducible and accurate for showing the behavior of molecules in polar/non-polar system.[20-27] Moreover, many calculated methods were used in order to predict the log P_{OW} value.[24,28,29]

Chromatographic parameter of lipophilicity, log k_w, obtained by extrapolation to pure water is calculated using the linear equation:[30]

$$\log k = \log k_w - S\varphi \quad (1)$$

where log k_w is the retention coefficient for pure water, S is the slope of the regression line, φ is the concentration expressed as molar fraction of organic solvent and water.

The R_M parameter determined in TLC is an analogous with log k value and can be combined with modifier concentration:

$$R_M = R_{MW} - S\varphi \quad (2)$$

where the definition of R_{MW}, S and φ are the same as in equation 1.

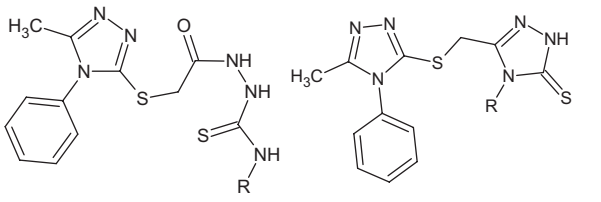
The aim of this work is the comparison the log P_{OW} of thiosemicarbazide derivatives and their cyclic analogues 1,2,4-triazole-3-thiones, which can be used as potential drugs, determined by RP-HPLC and RP-TLC methods with the calibration curve technique with the log P values calculated using known algorithms (milogP, ALOGPs, AClogP, AlogP, MLOGP, KOWWIN, XLOGP2, XLOGP3).[24,25,31] According to the Organisation for Economic Co-operation and Development (OECD) guidelines, in the chromatographic method selecting the appropriate reference compounds were required.[31] The influence of the structure of analyzed derivatives on the retention is also discussed. The different chromatographic behavior of both groups of compounds (linear and cyclic derivatives) were compared.

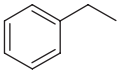
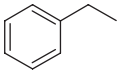
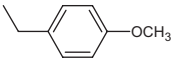
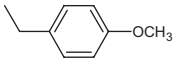
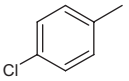
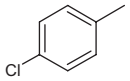
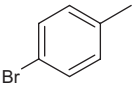
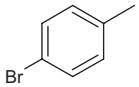
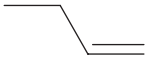
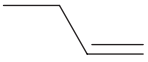
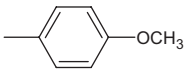
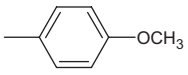
Experimental

Materials

Thiosemicarbazide derivatives and their cyclic analogues 1,2,4-triazole-3-thiones (Table 1) were synthesized in the laboratory at the Department of Organic Chemistry, Medical University of Lublin.[1] Methanol LiChrosolv (Merck, Darmstadt, Germany) for liquid chromatography grade and bidistilled water were used as mobile phase components.

Table 1. List of compounds investigated



No.	R	No.	R
1		**10**	
2	C_2H_5-	**11**	C_2H_5-
3		**12**	
4		**13**	
5		**14**	
6		**15**	
7		**16**	
8		**17**	
9		**18**	

High performance liquid chromatography

All HPLC experiments were performed using a chromatograph equipped with Elite LaChrom L-2130 gradient pump (Hitachi-Merck, Darmstadt, Germany), SPD-10AVP UV-VIS detector (Shimadzu, Kyoto, Japan) and Rheodyne 7725i valve with 20 µL loop.

20 µL of each sample (0.1% solution) was applied into the chromatographic column (RP-18 Waters Symmetry, 15 cm length, 4.6 mm i.d., 5 µm particle size) using a Hamilton syringe (Hamilton, Bonaduz, Switzerland). Mobile phases were degassed by use of built-in membrane degasser. Chromatograms were developed at flow rate of 1.0 mL min^{-1} in isocratic mode using various concentrations of modifier in binary polar mobile phases: methanol ranges

were 40-65% (v/v) changed by 5% *per* step (Table 2). Chromatograms were detected at 254 nm. All experiments were repeated in triplicate and the final results were their arithmetic mean.

Dead time was measured by use of uracil (Calbiochem-Merck, Darmstadt, Germany). All the experiments were performed at ambient temperature.

Thin layer chromatography

Thin layer chromatography was performed on 10 × 10 cm TLC plates coated with RP-18_{254} using methanol-water mixtures as mobile phases (Table 2). 0.1% of the methanolic solutions were applied on the plates and they were developed to a distance of 9 cm at room temperature in horizontal chambers (Chromdes, Lublin, Poland). The plates were not evaporated before the development. After drying in air, the chromatograms were visualized at a wavelength of 254 nm. Each experiment was performed three times.

Table 2. The concentrations of used eluents, n-number of points

Method	Eluent system	Concentration / %	Number of compound	n
TLC	Methanol/water	45-80	**1**-**18**	8
HPLC	Methanol/water	40-65	**10**	6
		40-60	**1**-**6**, **16**-**18**	5
		45-65	**7**, **8**, **9**, **11**-**15**	5

Standard solutes

According to the OECD guideline, in order to correlate the measured capacity factor log k of a standard compound with its log P_{OW}, a calibration graph using at least six points has to be established. It is preferable that the appropriate reference compounds should be structurally related to the test substances. Eight compounds were selected as standard solutes with optimal range of log P_{OW} units (0.9 to 4.9). The following standard substances were selected (the log P_{OW} values in brackets): aniline (0.9), 2-hydroxyquinoline (1.26), bromobenzene (3.0), naphthalene (3.6), propylbenzene (3.7), biphenyl (4.0), butylbenzene (4.6), pentylbenzene (4.9).

The standard compounds with known log P_{OW} were analyzed under the same chromatographic conditions as the tested substances (RP-HPLC and RP-TLC) in order to determine the lipophilicity parameter (log P_{HPLC} and log P_{TLC}).

All experiments were repeated in triplicate and the final results were their arithmetic mean.

log P calculation

The log P values of 1,2,4-triazole-3-thione derivatives were calculated using the two computer programs.[32-34]

Results and Discussion

The structures of analyzed compounds are presented in Table 1 and they were divided into two groups. The linear thiosemicarbazide derivatives (first nine compounds) and their cyclic analogues (remaining compounds) have the same substituents and they differ the lack of one molecule of water for cyclic analogues 1,2,4-triazole-3-thiones. The retention parameters were determined using the RP-HPLC and RP-TLC chromatographic systems. Mobile phases compositions for both chromatographic methods are presented in Table 2. Both the log k and the R_M values decreased linearly with the increasing of methanol concentration in the mobile phase. The parameters of the linear equation for HPLC and TLC methods are presented in Table 3. The high correlation coefficients ($r > 0.98$) and small values of the standard errors of estimate (< 0.1) were indicated that all equations obtained were highly significant. The chromatographic lipophilicity parameters (log k_W, R_{MW}) were obtained from equations 1 and 2 by extrapolation to pure water. In all cases, the value of log k_W is always higher than R_{MW} (Table 3). Probably, the differences in the log k_W and R_{MW} values are associated with "thin-layer effect" and the presence of apparent effluent front.

The correlation chart between the log k_W and the R_{MW} values was prepared and this relationship is described by the following equation:

$$R_{MW} = (1.1098 \pm 0.064) \log k_w - (0.8757 \pm 0.222)$$
$$r^2 = 0.9488,\ n = 18,\ F = 296.7,\ s_e = 0.158 \quad (3)$$

High value of correlation coefficient confirms the similarity of both experimental methods (RP-HPLC and RP-TLC).

The differences between the chromatographic lipophilicity parameters, log k_W obtained for second (cyclic analogues) and first (linear derivatives) groups ($\Delta \log k_{WB-A}$) were calculated (Table 4) and they are in the range from 0.021 to 0.508 (average value = 0.272). In the case of the TLC method, these differences are in the range from 0.045 to 0.597 (average value = 0.272). Negative values of were obtained for *p*-chlorophenyl and *p*-bromophenyl in HPLC and TLC methods. High value of chromatographic lipophilicity parameters log k_W and R_{MW} for compounds **3** and **12** (with cyclohexyl substituent) were observed

(Table 3). Low differences between the values of R_{MW} for **3** and **12** were noted (Table 4).

The structure of the first group is different from the second group of the lack of water molecule. The elimination of water from a molecule reduces its lipophilicity as well as absolute values of the specific hydrophobic surface, and the ratio of the intercept (log k_W) to the slope (–S) of the compound is constant in both groups.[35] These results are in accordance with the fragmental method used for log P calculations.[36]

The lowest value of log k_W and R_{MW} was obtained for compounds **2**, **8**, **11** and **17**. There are substances containing the ethyl group (**2**, **11**) and but-1-ene group (**8**, **17**) in their structure. Compounds **5** and **9** differ in chain length (one methylene group), similarly the substances **14** and **18**. This small difference in structure slightly affects the value of the parameters of lipophilicity, which are higher for compounds with longer carbon chain (Table 3).

A significant influence of the structural differences was observed for substances **1**, **10** and **3**, **12**, which contain the phenyl and the cyclohexyl groups, respectively. Higher value of lipophilicity parameters (Table 3) was obtained for compounds **3** and **12** (the average of differ for log k_W is 0.8861 and for R_{MW} is 1.2462). Moreover, the change of halogen group for compounds **6**, **7**, **15** and **16** did not significantly affect the change in value of lipophilicity.

Comparing the log k_W and R_{MW} values of two groups of analyzed compounds (the linear thiosemicarbazide derivatives and their cyclic analogues), some differences have been observed. Generally, the slightly higher values of these factors were obtained for second group of compounds (**10**-**18**) in most of the cases (Table 3). The increase of the lipophilicity is probably due to the presence of the additional triazole ring, which changes the position of the whole molecule in space. The exceptions are the substances **6** and **7**, for which the log k_W and R_{MW} values are slightly higher than for compounds **15** and **16**. The *p*-chlorophenyl substituent is presented in the structure of compounds **6** and **15** and the *p*-bromophenyl substituent is presented in the structure of compounds **7** and **16**. The presence of halogen substituents affects the chromatographic behavior of the whole molecule. The proximity of chlorine and bromine (free electron pairs) can cause changes in stereochemistry and different interactions of the molecule with the stationary and mobile phases. A linear relationship between the intercept and slope from equations 1 and 2 for the used mobile phase, is one of the basis features of chromatographic determination of the lipophilicity of closely related compounds.[37] In this study, the

Table 3. Parameters of the equations 1 and 2 for methanol-water system

Compound	HPLC						TLC					
	log k_W	–S	r	n	s_e of estimation	F	R_{MW}	–S	r	n	s_e of estimation	F
1	3.0352	5.3645	0.9983	5	0.03	858.39	2.3411	3.6543	0.9907	8	0.07	319.45
2	2.4426	4.7697	0.9969	5	0.03	481.84	1.7865	3.0208	0.9916	8	0.05	354.31
3	3.9497	5.9981	0.9966	5	0.05	443.35	3.6405	4.9825	0.9942	8	0.07	509.90
4	3.5468	5.7178	0.9990	6	0.03	1994.1	3.0721	4.4441	0.9943	8	0.06	520.93
5	3.5811	5.9281	0.9984	6	0.06	1272.8	3.0796	4.4790	0.9920	8	0.08	370.27
6	4.1295	6.2585	0.9965	5	0.05	428.12	3.6564	5.0826	0.9941	8	0.07	503.42
7	3.9801	6.1719	0.9978	5	0.04	672.51	3.4743	4.9134	0.9929	8	0.08	420.95
8	2.3304	4.2846	0.9980	5	0.02	745.62	1.9951	3.2856	0.9912	8	0.06	335.40
9	2.8211	4.9485	0.9975	5	0.03	586.04	2.4412	3.7471	0.9876	8	0.08	238.30
10	3.1132	5.5839	0.9980	5	0.03	746.02	2.3861	3.6727	0.9909	8	0.07	325.44
11	2.6314	5.0563	0.9989	5	0.02	1414.1	1.9261	3.0708	0.9928	8	0.05	411.24
12	3.9708	5.9570	0.9968	5	0.04	471.74	3.5791	4.8016	0.9974	8	0.05	1135.2
13	3.7650	5.7924	0.9907	5	0.07	158.52	3.5827	4.9120	0.9940	8	0.07	491.85
14	3.9828	6.1814	0.9967	5	0.05	459.76	3.6769	5.0297	0.9959	8	0.06	724.80
15	3.9537	6.0530	0.9971	5	0.04	520.93	3.4559	4.7799	0.9959	8	0.06	734.59
16	3.8291	5.9848	0.9970	5	0.04	493.84	3.2870	4.6106	0.9940	8	0.07	492.75
17	2.8379	5.1475	0.9994	5	0.02	2362.2	2.2655	3.5013	0.9911	8	0.06	33.17
18	3.3071	5.7647	0.9982	5	0.03	833.35	2.5212	3.6790	0.9859	8	0.08	208.81

logk_w: retention coefficient for pure water; S: slope of the regression line; r: correlation coefficient; n: number of points; s_e: standard error of estimation; F: statistica F.

Table 4. The lipophilicity differences between the 3H-1,2,4-triazol-3-thiones and the corresponding semicarbazides ($\Delta\log k_{WB-A}$, ΔR_{MWB-A}) obtained by two chromatographic systems

	$\Delta\log k_{WB-A}$	ΔR_{MWB-A}
1_A (10_B)	0.078	0.045
2_A (11_B)	0.189	0.140
3_A (12_B)	0.021	–0.061
4_A (13_B)	0.218	0.511
5_A (14_B)	0.402	0.597
6_A (15_B)	–0.176	–0.201
7_A (16_B)	–0.151	–0.187
8_A (17_B)	0.508	0.270
9_A (18_B)	0.486	0.080

good correlation obtained between the intercept ($\log k_W$, R_{MW}) and slope (S) confirms the suitability of these systems for estimation of the lipophilicity of thiosemicarbazide derivatives and their cyclic analogues. The linear correlation is described by the following equations:

$$\log k_w = (0.9043 \pm 0.063)S - (2.5341 \pm 0.217)$$
$$r^2 = 0.9282,\ n = 18,\ F = 206.8,\ s_e = 0.155 \quad (4)$$

$$R_{MW} = (1.0775 \pm 0.024)S - (1.08679 \pm 0.071)$$
$$r^2 = 0.9922,\ n = 18,\ F = 2047.6,\ s_e = 0.067 \quad (5)$$

The rule of 5" developed by Lipiński *et al.*[38] predicts that poor absorption or permeation is more likely when there are molecules (drug-like) that have more than 5 H-bond donors, 10 H-bond acceptors in their structure, the molecular weight (MWT) is greater than 500 and the calculated log P (C log P) is greater than 5 (or M log P > 4.15).[38] In our work, newly synthesized thiosemicarbazides and their cyclic analogues 1,2,4-triazol-3-thiones were in agreement with the rule of five claimed by Lipiński *et al.*[38] (Table 5).

Reversed phase high performance liquid chromatography and reversed phase thin layer chromatography were also used in order to determine experimentally octanol-water partition coefficients ($\log P_{HPLC}$ and $\log P_{TLC}$-parameters). The measurements were conducted according with the OECD guidelines.[31] Similar studies were carried out in many works.[24-27] In this study, eight compounds have been selected (see Experimental section) as reference compounds from the Recommended Reference Compounds list published by the OECD.[31] The determination of $\log P_{OW}$ by HPLC and TLC methods is based on the linear relationship between the chromatographic retention parameters ($\log k$ and R_M) and the octanol-water partition coefficient determined by shake-flask method for selected standard solutes.

Table 5. Data applied for the rule of 5" for tested compounds

Compound	Molecular weight	H bond acceptors	H bond donors	$\log P_{HPLC}$	$\log P_{TLC}$
1	398.517	7	3	1.89	0.85
2	350.473	7	3	1.37	0.55
3	404.565	7	3	3.04	1.86
4	412.544	7	3	2.48	1.39
5	442.57	8	3	2.37	1.29
6	432.962	7	3	3.08	1.76
7	477.413	7	3	2.88	1.50
8	362.484	7	3	1.74	0.61
9	428.543	8	3	1.95	1.02
10	380.502	6	1	1.77	0.91
11	332.458	6	1	1.37	0.85
12	386.55	6	1	3.15	2.13
13	394.529	6	1	2.9	1.86
14	424.555	7	1	2.86	1.86
15	428.974	6	1	2.97	1.76
16	473.425	6	1	2.79	1.55
17	344.469	6	1	1.72	0.96
18	410.528	7	1	1.96	1.39

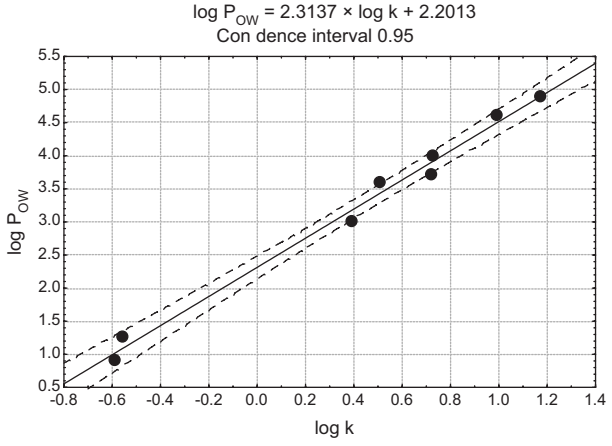


Figure 1. Calibration graph for standard solutes for RP-HPLC method.

In the case of RP-HPLC method, the best selectivity was obtained with methanol-water (60:40, v/v) and this mobile phase was chosen for the determination of $\log P_{OW}$. Linear calibration equation (Figure 1) between log k values and their literature $\log P_{OW}$ for standard compounds looks as follows:

$$y = (2.3137 \pm 0.07)x + (2.2013 \pm 0.09)$$
$$r = 0.9946 \pm 0.04,\ n = 8,\ F = 552.5,\ s_e = 0.16 \quad (6)$$

Next, the log k values of the studied substances were substituted into equation 6 to calculate the partition coefficient obtained for HPLC method ($\log P_{HPLC}$).

Similar procedures were carried out for RP-TLC method. In this case, the mobile phase containing 70% methanol in water (%, v/v) was proved to be the best selectivity system. Linear relationships between the R_M and the log P_{OW} was obtained (Figure 2):

$$y = (2.8458 \pm 0.12)x + (1.6296 \pm 0.09)$$
$$r = 0.9950 \pm 0.04,\ n = 8,\ F = 599.9,\ s_e = 0.16 \quad (7)$$

The determination of linear relationships between experimental lipophilicity parameters (log P_{HPLC} and log P_{TLC}) and calculated log P values is a necessary step

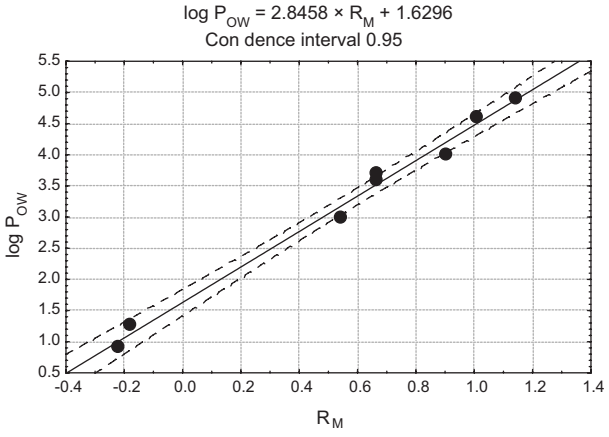


Figure 2. Calibration graph for standard solutes for RP-TLC method.

for QSAR analysis.[25] In our work, these correlations were performed separately for two groups of tested compounds and the extrapolated log k_W and R_{MW} values and experimentally established log P_{HPLC} and log P_{TLC} values were compared with calculated log P (log P_{calc}). Generally, in the case of the RP-HPLC method, high values of correlation coefficient were obtained for first group of analyzed compounds (thiosemicarbazide derivatives) ($0.8244 < r < 0.9808$) in comparison with their cyclic analogues ($0.6615 < r < 0.9456$). The weaker correlations were obtained for RP-TLC method, where for the first group of compounds, the partition coefficient was in the range: $0.7770 < r < 0.9795$ and for second group: $0.6183 < r < 0.9030$. The best results for correlations between the experimental and calculated partition coefficients were obtained for relationships between extrapolated (log k_W and R_{MW}), experimental (log P_{HPLC} and log P_{TLC}) parameters and calculated partition coefficient.

In order to better illustrate these correlations, the unscaled principal component analysis (PCA) with loadings interpretation was used. The experimental data (log k_W, R_{MW}, log P_{HPLC} and log P_{TLC}) from Tables 3 and 5 and the calculated log P parameters (from Table 6) were grouped as data matrix and they were analyzed using PCA, based on covariance matrix (unscaled PCA) using the Statistica 8 (StatSoft Inc. 2007) and results are presented in Figure 3. The experimental data was used as supplementary data

Table 6. Values of log P_{calc} parameter calculated by computer programs. The values of log P_{HPLC} and log P_{TLC} are presented in Table 5

No.	milogP	ALOGPs	AClogP	ALOGP	MLOGP	KOWWIN	XLOGP2	XLOGP3
1	1.86	2.64	2.07	3.23	3.70	2.39	4.15	3.17
2	1.17	1.72	0.93	2.01	2.67	1.09	3.01	1.98
3	2.70	2.94	2.08	3.52	3.66	2.86	4.41	3.42
4	2.20	2.62	1.86	3.24	3.67	2.30	4.29	3.11
5	2.25	2.76	1.75	3.22	3.41	2.38	4.21	3.08
6	2.54	3.39	2.68	3.90	4.20	3.03	4.77	3.80
7	2.67	3.36	2.77	3.98	4.32	3.28	4.95	3.86
8	1.44	1.68	1.10	2.28	2.85	1.44	3.20	2.26
9	1.91	2.78	1.97	3.22	3.45	2.47	4.06	3.14
10	2.22	3.26	3.56	4.11	5.14	5.50	5.13	3.67
11	1.32	2.47	2.73	2.88	4.11	3.86	3.99	2.48
12	2.85	3.83	3.89	4.39	5.09	5.64	5.39	3.92
13	2.54	3.50	3.67	4.12	5.10	5.07	5.28	3.61
14	2.60	3.50	3.56	4.10	4.84	5.16	5.19	3.58
15	2.90	4.05	4.28	4.78	5.60	5.72	5.90	4.23
16	3.03	4.15	4.37	4.87	5.71	5.96	6.07	4.30
17	1.59	2.76	2.90	3.15	4.29	4.21	4.18	2.76
18	2.28	3.34	3.45	4.09	4.61	5.58	5.05	3.64

(in Figure 3 they are marked with squares). The strongest correlations between the experimental log P factors and parameters were confirmed.

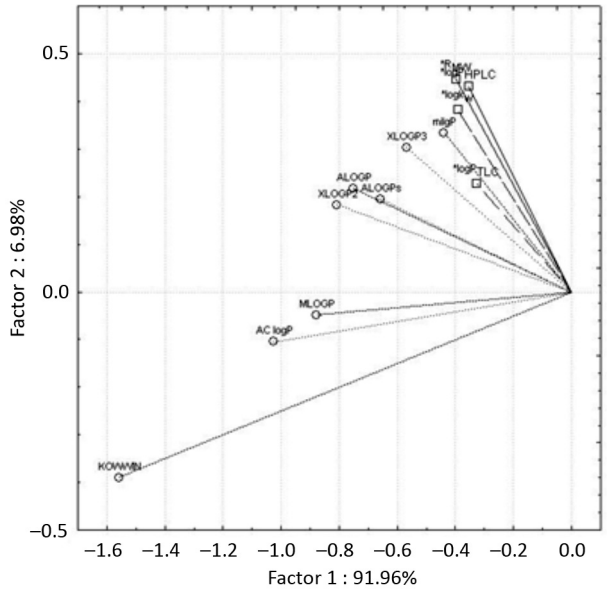


Figure 3. Experimental and calculated data projection on the plane determined by two first principal components.

Obtained results confirm that the chromatographic methods used to measure the lipophilicity of the thiosemicarbazides and their cyclic analogues 1,2,4-triazol-3-thiones are valid and suitable.

Conclusions

Values of the relative lipophilicity parameters log k_W and R_{MW} were converted into log P_{HPLC} and log P_{TLC} values by use of the a calibration graph obtained by use of ten standard solutes. This study shows that aniline, 2-hydroxyquinoline, bromobenzene, naphthalene, propylbenzene, biphenyl, butylbenzene and pentylbenzene are useful as reference substances for the determination of partition coefficient octanol-water using HPLC and TLC methods.

The influence of the structure of the thiosemicarbazides and their cyclic analogues 1,2,4-triazol-3-thiones on the value of lipophilicity was observed.

Moreover, the good correlation between the intercept (log k_W, R_{MW}) and slope (S) confirms the suitability of these systems for estimation of the lipophilicity of thiosemicarbazide derivatives and their cyclic analogues.

The best correlations between the experimental (or extrapolated) partition coefficients and milogP parameters were obtained. Generally, higher values of partition coefficient for these relationships were obtained for RP-HPLC method.

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