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Article

Establishing Dependences between Different Lipophilic Parameters of New Potentially Biologically Active *N*-Substituted-2-Phenylacetamide Derivatives by Applying Multivariate Methods

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Lipophilicity, a very important parameter in the potential biological activities of molecules, was investigated for newly synthesized *N*-substituted-2-phenylacetamide derivatives. The determination was carried out in two ways: first experimentally, by applying thin-layer chromatography (TLC) on reversed-phase TLC (RPTLC) RP18F254s in the presence of one protic (methanol) and one aprotic solvent (acetonitrile) and then mathematically, by using different software packages. The intercept of the linear dependence between volume fractions of the organic solvent and the retention parameters obtained by TLC is known as the retention chromatographic constant, R_M^0, while the slope represents the *m* value. In order to establish the dependences between the partition coefficient, log *P* as the standard measure of lipophilicity and the alternative lipophilic parameters obtained experimentally by TLC, R_M^0 and *m* values, linear regression analysis and multivariate methods, cluster analysis (CA) and principal component analysis (PCA), were used. All applied methods gave approximately similar results. Although there is a linear dependence between the two chromatographic parameters, the retention constant, R_M^0, and the *m* values, only R_M^0 shows suitable similarity with the standard measure of lipophilicity of the investigated *N*-substituted-2-phenylacetamide derivatives at the given conditions. The existence of this resemblance proves that the chromatographic retention constant, R_M^0, obtained by RPTLC could be successfully used for the description of lipophilicity of investigated compounds. On the other hand, the results confirmed that the applied linear regression analysis and the multivariate analysis (CA and PCA) have the ability to compare lipophilic parameters of the investigated phenylacetamide derivatives obtained in different ways.

Introduction

The recognition, examination and synthesis of pharmacophore are important and long-lasting parts of a complex process in design of drugs. Contemporary research studies have found that the amide group is present and active in different newly discovered bioactive molecules. A significant place among the amide derivatives is occupied by phenylacetamides, first known by their analgesic and antipyretic properties (1, 2) and over time by their applications; phenylacetamides are found in the treatment of various diseases. Many of them have been recognized as effective agents in the treatment of rheumatoid arthritis and tuberculosis (3, 4). In addition, large investments were made to examine the possibility of the application of phenylacetamides as anticonvulsants, antidepressants and antipsychotics (5–9), but it is without a doubt that the greatest interest of scientists was stirred by their antioxidant and anticancer activity (10–13). Besides the abovementioned properties, phenylacetamides are a proven bactericides, fungicides and anthelmintics (14–17).

Given that the type, the intensity of the activities and the efficiency of phenylacetamides depend on the nature of the substituents attached to the basic molecule, for the research it is necessary to know and establish links between activities, structure and physicochemical characteristics of the potentially active substance. The selection of structural parameters that allow a better understanding of the behavior of the active substance in a biological medium is provided by applying the quantitative structure–activity relationship (QSAR) model. Lipophilicity is the most frequently used molecular descriptor in QSAR studies, which determines the transport of compound through the biological system (18). This parameter points to the biological activity of molecules uniting its pharmacodynamics, pharmacokinetics and possible toxicity (19). It is widely accepted that lipophilicity can be quantitatively expressed as log *P* (the logarithm of the ratio of the concentrations of solute in a saturated 1-octanol–water system) (20–22). In addition to the standard measure of lipophilicity, in practice the alternative measure of lipophilicity, chromatographic retention constant, R_M^0, obtained by RPTLC (23–29) is often used. This retention chromatographic constant, R_M^0, can be determined as an intercept of the linear dependence established between the volume fractions of the organic solvent and the retention parameters. The quantitative structure–retention relationship model enables the linking structure and the retention in order to obtain a more complete picture of the properties of the investigated compounds (18, 30).

The subject of this study was examining the retention behavior of the selected *N*-substituted-2-phenylacetamide derivatives and establishing a correlation between the experimental and software obtained lipophilicity parameters. The correlation was performed by classical linear regression and also by using two multivariate methods, cluster analysis (CA) and principal component analysis (PCA).

Experimental

The structures of the investigated compounds are presented in Figure 1.

Instrumentation and reagents

Solutions (2 mg mL^{-1}) for chromatographic investigations were prepared by dissolving compounds in ethanol. These solutions

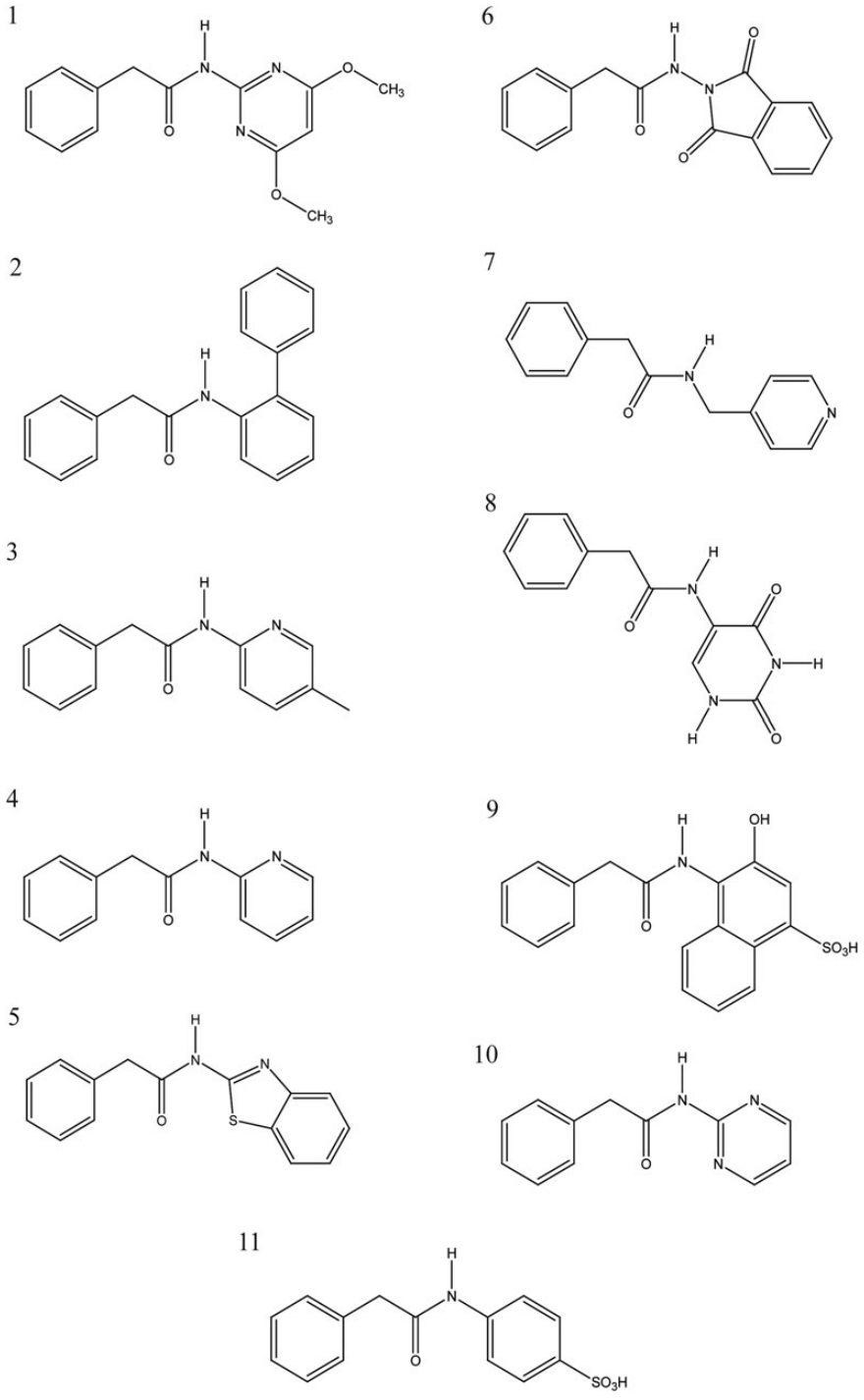

Figure 1. Structures of the investigated *N*-substituted-2-phenylacetamides.

(0.2 μL) were spotted on the stationary phase (RPTLC C_{18}/UV_{254} plates, Macherey-Nagel). The plates were developed in unsaturated chambers by the ascending technique at the room temperature with aqueous solutions of two organic modifiers: methanol ($\varphi = 0.34–0.52$, v/v) and acetonitrile ($\varphi = 0.36–0.52$, v/v). After developing, the dried plates were examined in UV light at $\lambda = 254$ nm. At least three chromatograms were developed for each solute–solvent combination and the R_f values were averaged.

Methods

The obtained experimental data were processed by a software package Origin, version 6.1. Standard lipophilicity values, log *P*, were calculated using the Virtual Computational Chemistry Laboratory (VCCLAB; http://www.vcclab.org, accessed 14 May 2011). The CA and PCA procedures were performed by the Statistica v.12 software (StatSoft Inc., Tulsa, OK, USA).

Results

Determination of the retention behavior of the investigated N-substituted-2-phenylacetamide derivatives

Thin-layer chromatography (TLC) on reversed-phase RP 18 F254s HPTLC was used in order to designate the retention behavior of the tested *N*-substituted-2-phenylacetamide derivatives experimentally, in the presence of methanol as a protic solvent and acetonitrile as an aprotic solvent. The chromatographic retention behavior of the newly synthesized *N*-substituted-2-phenylacetamide derivatives in RPTLC is presented in Table I.

More information about the effects of the mobile phase on chromatographic retention behavior of the studied *N*-substituted-2-phenylacetamide derivatives could be obtained by changing the amount of organic solvent in the mobile phase. Based on the experimentally determined R_f values for each composition of the mixture, the R_M value was calculated using the following equation:

$$R_M = \log(1/R_f - 1). \quad (1)$$

The value of the retention constant, R_M^0, can be determined by extrapolating R_M values at 0% of organic modifier by using the following linear relationship:

$$R_M = R_M^0 + m\varphi, \quad (2)$$

where φ is the volume fractions of the organic solvent in the mobile phase and m (slope of TLC equation) is the shift in R_M value caused by unit change of the organic modifier volume fraction in the mobile phase and R_M^0 (intercept) is the retention constant. The results for the obtained correlation are presented in Table II. The linear dependences in the selected field of work for all tested organic modifiers are confirmed with the high values of the correlation coefficients, r.

It is apparent from the data in Table II that the obtained R_M^0 values and the absolute value of m increase with increasing hydrophobicity of all tested phenylacetamides.

Therefore, these two constants were correlated. Figures 2 and 3 show this dependence between these two chromatographic parameters in methanol and acetonitrile, respectively. The obtained equations of these linear dependences are given in Table III.

Determining chromatographic retention constant, R_M^0, by RPTLC is important because this constant is widely used as a measure of lipophilicity, instead of reference lipophilicity parameter, log P (23–29). In order to test these facts, the obtained values of both the chromatographic parameters, R_M^0 and m, were compared with the standard measure of lipophilicity using the linear regression analysis and two methods of multivariate analysis.

Table I

R_f Values of *N*-Substituted-2-Phenylacetamide Derivatives in the C-18 RP-TLC Stationary Phase in a Variety of Mobile Phases Containing 40% Organic Modifier and 60% Water

Compound	Methanol	Acetonitrile
1	0.13	0.29
2	0.06	0.15
3	0.12	0.29
4	0.19	0.30
5	0.05	0.20
6	0.29	0.49
7	0.21	0.31
8	0.40	0.55
9	0.65	0.67
10	0.35	0.51
11	0.78	0.89

Table II

Extrapolated R_M^0 Values, Slope, m, Correlation Coefficients, r, and the Standard Deviation of Estimation, SD, of TLC Equations $R_M = R_M{}^0 + m\varphi$

Compound	Water–methanol				Water–acetonitrile			
	R_M^0	m	r	SD	R_M^0	m	r	SD
1	2.243	−3.524	0.971	0.041	1.724	−3.294	0.997	0.051
2	2.375	−3.243	0.935	0.145	2.570	−4.487	0.996	0.057
3	2.230	−3.421	0.998	0.019	1.656	−3.114	0.996	0.043
4	1.806	−2.931	0.997	0.065	1.531	−3.061	0.998	0.036
5	2.705	−3.736	0.994	0.089	2.281	−4.096	0.995	0.076
6	1.582	−3.041	0.992	0.096	1.558	−3.335	0.994	0.085
7	1.709	−3.190	0.997	0.091	1.449	−2.982	0.998	0.043
8	0.945	−2.992	0.988	0.097	0.659	−2.416	0.996	0.048
9	0.895	−2.928	0.999	0.011	0.494	−2.760	0.988	0.079
10	1.154	−2.211	0.991	0.088	0.941	−2.418	0.996	0.055
11	0.782	−3.384	0.998	0.041	−0.129	−1.914	0.998	0.031

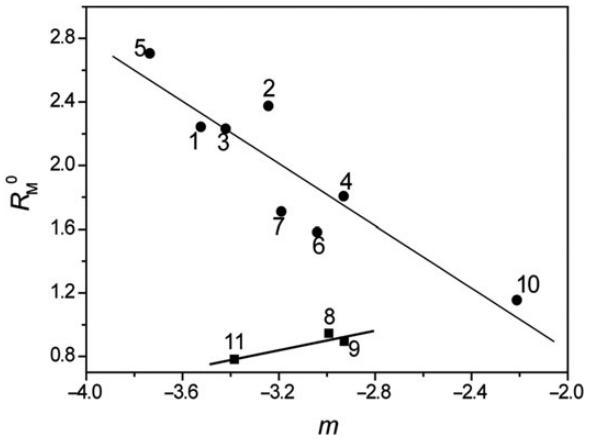

Figure 2. Relationship between R_M^0 and m, obtained in methanol.

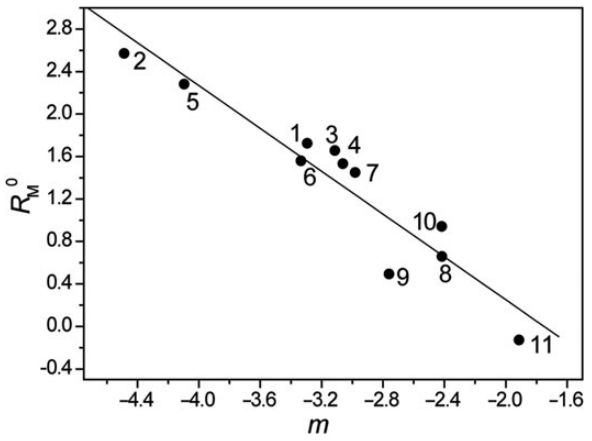

Figure 3. Relationship between R_M^0 and m, obtained in acetonitrile.

Table III
Equations of Relationship between Intercept, R_M^0, and Slope, m, in All Applied Solvents

Modifier	Equation	r	SD
Acetonitrile	$R_M^0 = -1.766 - 1.008m$	0.945	0.273
Methanol[a]	$R_M^0 = -1.117 - 0.978m$	0.906	0.229
Methanol[b]	$R_M^0 = 1.826 - 0.307m$	0.907	0.050

[a]Correlation for compounds containing non-polar substituent $-R$.
[b]Correlation for compounds containing polar substituent $-R$.

Table IV
Mathematical log P Values of Investigated N-Substituted-2-Phenylacetamides

Compound	AC log P	AB log P	mi log P	A log P	M log P	"Kowwin"	x log P
1	2.42	2.38	2.32	2.44	2.89	2.62	1.92
2	4.55	4.47	4.67	4.17	4.33	4.01	3.61
3	2.50	2.37	2.47	2.52	2.69	2.73	2.32
4	2.27	1.96	2.02	2.04	2.43	2.18	2.20
5	3.77	3.06	3.48	3.28	3.26	3.73	3.48
6	1.34	2.26	2.41	1.73	2.92	1.93	2.00
7	1.58	1.29	1.33	1.51	1.61	1.53	1.56
8	−0.05	−0.38	0.23	−0.49	0.73	0.12	0.01
9	1.34	1.85	0.73	2.25	2.35	0.35	2.34
10	1.67	1.54	1.52	1.39	1.75	1.54	1.31
11	0.46	0.34	−0.09	1.61	2.01	−0.35	1.81

Mathematical determination of the lipophilicity of N-substituted-2-phenylacetamide derivatives

Using the VCCLAB, the standard measure of lipophilicity and partition coefficients, log P, of investigated phenylacetamides were calculated (http://www.vcclab.org, accessed 14 May, 2011). The obtained values of log P are presented in Table IV.

The correlation between the standard measure of lipophilicity, log P, and chromatographic parameters, R_M^0 and m

In order to prove the previously assumed fact that chromatographic parameters, R_M^0 and m, can be used to express and determine lipophilicity, standard measures of lipophilicity, log P calculated in different ways and experimentally determined lipophilicity, chromatographic retention constants, R_M^0 and values of m, were correlated using a linear regression analysis and different multivariate analysis. For identification and separation of the most important information from the obtained experimental data in chromatographic analysis, CA and PCA are the most commonly used multivariate methods (31–38).

The linear regression analysis results from correlation between chromatographic retention parameters, R_M^0 and m, obtained in acetonitrile and one of the calculated partition coefficients, AB log P, are presented in Figures 4 and 5, respectively.

In Table V, the correlation matrix is presented, obtained by the correlation between various log P and R_M^0 for the investigated compounds using linear regression analysis.

The correlation of measured parameters of lipophilicity in addition to the method of linear regression was also performed by applying two multivariate methods: CA and PCA.

The data matrix prepared for the CA and PCA determination consists of columns (variables) that correspond to the lipophilicity calculated in different ways, and of rows (cases) that correspond to the phenylacetamide derivatives. CA and PCA were applied after standardization, during which the column values were subtracted from each matrix element and each matrix element was divided by the standard deviation of each column. The Euclidean distance was used in CA as the measure of dissimilarity between objects, while for testing the linkage measure the Ward's linkage method was applied. Dendrogram obtained by using CA for different parameters of lipophilicity is presented on Figure 6.

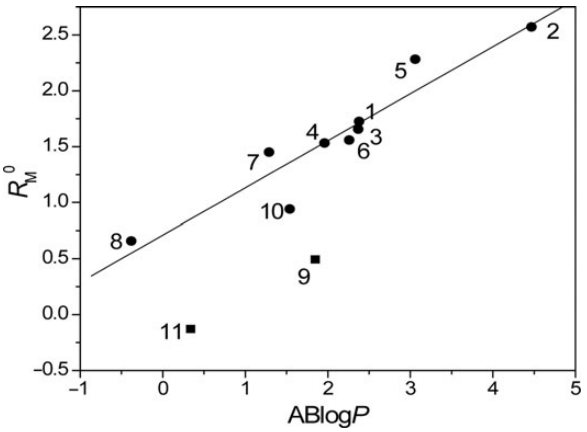

Figure 4. Relationships between AB log P values and retention constant, R_M^0, in acetonitrile.

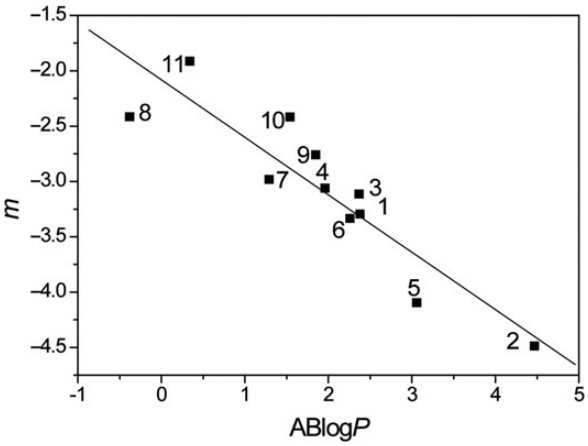

Figure 5. Relationships between AB log P values and chromatographic parameters, m, in acetonitrile.

Table V
Correlation Matrix between Different log P and Chromatographic Parameters R_M^0 and m: $Y(R_M^0 \text{ or } m) = a + b \log P$

Modifier	AC log P	AB log P	mi log P	A log P	M log P	"Kowwin"	x log P
R_M^0 (ACN)[a]	0.941	0.941	0.957	0.957	0.939	0.964	0.962
R_M^0 (MET)[a]	0.875	0.816	0.822	0.891	0.815	0.927	0.892
m (ACN)	0.905	0.906	0.953	0.788	0.843	0.917	0.782
m (MET)	–	–	–	–	–	–	–

[a]From the correlation compounds 9 and 11 are excluded.

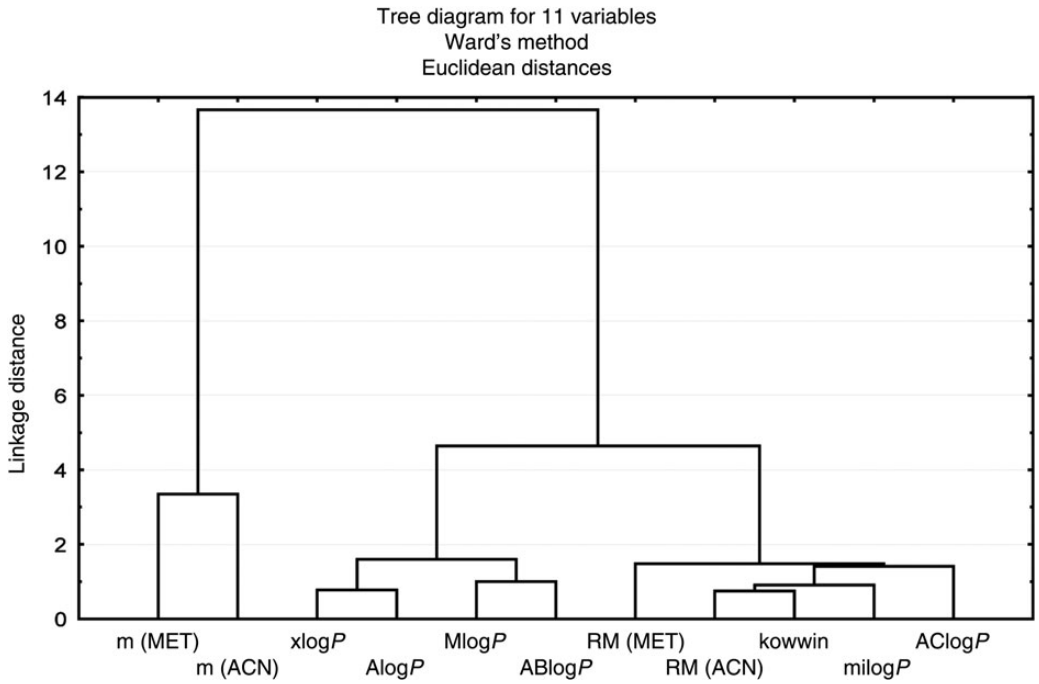

Figure 6. Dendrogram of the lipophilicity parameters in the space of 11 measured values.

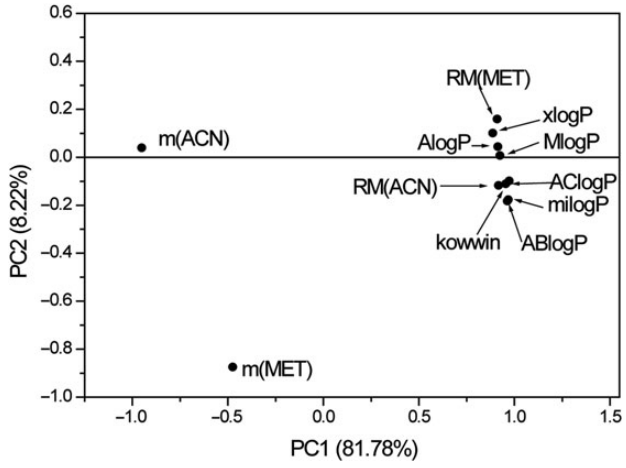

Figure 7. Loading plots as a result of PCA.

The second multivariate method, which was used for comparing different lipophilic parameters, is the PCA. The essence of PCA is to disassemble the original data matrix into several products of multiplication into loading (lipophilic parameters) and score (investigated compounds) vectors, whereby are obtained new variables so-called principal components (PCs).

By implementing the PCA onto the lipophilic data matrix, three PCs were gained that describe 97.22% (PC1 81.78%, PC2 8.22% and PC3 7.21%) of the total data variance.

The similarity between the analyzed lipophilic parameters calculated in different ways (variables) can be seen in the loading plot, which is obtained by applying the PCA procedures on the variables. The obtained loading plot for two dominant PC (PC1 against PC2) is presented in Figure 7.

Discussion

In order to establish dependences between the alternative lipophilic parameters obtained experimentally by TLC (R_M^0 and m) and the partition coefficient, log P as the standard measure of lipophilicity linear regression analysis and multivariate methods, CA and PCA, were used.

As the results in Table I show, the retention data of the investigated compounds obtained for the separation on the C-18 bonded silica gel in both mobile phases are generally of typical reversed-phase chromatographic behavior: the data depend on the nature of the used organic modifier as well as the chemical characteristics of the substituent $-R$ related to the nitrogen atom of the amide group. From the acquired results, it is evident that the selected organic solvent has a much smaller influence on the retention behavior of the compounds than the nature of the substituent. This can be explained by the different interaction that occurs between applied organic modifiers and the investigated amides during the chromatographic analysis. In the case of investigated compounds, two kinds of interactions are dominant: polar interactions of the amide group with polar mobile phase and interactions of less polar stationary phase with hydrophobic substituent $-R$.

As was expected, compounds with less polar substituents are more strongly retained in both used solvents than compounds that have polar $-R$. For example, compound 2 because of the presence of most non-polar biphenyl group has the strongest retention, in contrast to compound 11, which exhibits the lowest retention because of the presence of polar benzenesulfonic acid as a substituent related to the nitrogen atom of the amide group.

Molecules 3 and 4 differ in the substituent $-R$ in the presence of the methyl group, which leads to a greater retention of compound 3, and it is typical for reversed-phase chromatography. The presence of an additional $-CH_2$ group within compound 7

leads to its predictable faster movement through the stationary phase, or rather to a lesser retention in comparison to molecule 4.

By observing the obtained R_f values of the same compound in different organic modifiers, different R_f values were obtained for methanol and acetonitrile as modifiers. The R_f values for the water–acetonitrile system are larger than the corresponding data acquired for water–methanol system.

In reversed-phase chromatography, in which the solvation effect plays a very important role, the retention depends on the molecular structure of the solute; therefore, the obtained R_M^0 values are different for each compound (Table II). However, it is also known that R_M^0 depends on the nature of the organic modifier of the binary aqueous eluents employed in the RPTLC (39). As a result, different R_M^0 values were obtained for methanol and acetonitrile as modifiers. The R_M^0 values from water–methanol system are larger than the corresponding data determined for water–acetonitrile system.

The obtained values of slope m are also not the same for all investigated substances (Table II). The most important factors that influence the value of the slope m are the characteristics of the solute and its chemical structure—type and number of functional groups in the molecule, specific hydrophobic surface (40). For the examined group of compounds, m depends not only on the solvent applied as a component of the mobile phase, but also on a considerable extent of a specific interaction between solutes, stationary and mobile phase. As Figures 2 and 3 show, in both used solvents, the linear relationship between intercept, R_M^0, and slope, m, can be noticed. In contrast to the acetonitrile, the separation of the compounds in methanol occurs based on the polarity of the substituent $-R$ related to the nitrogen atom of the amide group. It is noticeable the linear grouping of compounds 8, 9 and 11, which as substituent $-R$ have very polar groups, from the second group that includes molecules 1–7 and 10, which as the substituent $-R$ have non-polar groups. Atypical behavior of compounds 8, 9 and 11 may occur due to different degrees of dissociation of the molecules (polar substituent) under applied conditions. This fact, at the same time, could be explained by the significantly greater sensitivity of methanol as a protic modifier to the strength of the interaction in the solute–stationary phase, which results in a better separation of compounds.

The obtained linear equation indicates that both R_M^0 values and m seem to be related to the same physicochemical factors and, therefore, are intercorrelated. Because of this, some authors have suggested that in addition to the retention constant R_M^0, the value of m can also be used as descriptors for predicting lipophilicity, and thus the biological activity of newly synthesized compounds (41–45).

The standard measure of lipophilicity, log P, was calculated using different software packages. As the data in Table IV show for the same compounds different values of the partition coefficient were obtained. This fact can be explained by various ways of calculation. Furthermore, compound 2 has the highest value of partition coefficient in the chromatographic analysis as well, and the lowest value in the majority cases was obtained for compound 8. The obtained values for most polar compounds differ from the experimental results, where the lowest lipophilicity was registered for compound 11.

In the case of the correlation between R_M^0 determined for acetonitrile and AB log P, as Figure 4 shows, a good linear dependence was registered between these two lipophilic parameters. Deviation from this correlation was registered in the case of compounds 9 and 11. Their atypical behavior was also registered and explained earlier. A similar distribution of compounds was registered in the case of all determined log P values and also in the case when methanol was used as modifier.

The results presented in Table V confirm that good linear relationships exist between the standard measure of lipophilicity, log P, and retention constant R_M^0 obtained by RPTLC. Better dependence between these lipophilic parameters was registered in a case when acetonitrile was used as modifier. The best correlation was registered between "kowwin", and the lowest similarity was observed between Mlog P and R_M^0 in both modifiers.

The existence of a good linear dependence between log P as a standard measure of lipophilicity of the examined compounds and R_M^0 confirms the fact that the chromatographic retention constants, obtained by thin-layer chromatography in the reversed phase in both modifiers that were used, can be successfully used as a measure of lipophilicity of the newly synthesized derivatives *N*-substituted-2-phenylacetamides.

When the correlation between m obtained for acetonitrile and AB log P is observed (Figure 5), a good linear dependence can be noticed, but without further separation of compounds based on chemical properties of substituent $-R$ (Table V). In the case when methanol was used as modifier, defined dependence between these two parameters could not be registered.

Based on the results of linear regression analysis, it can be concluded that the value of R_M^0 compared with the value of m is nevertheless more similar to the standard measure of lipophilicity, log P.

Using CA as indicated by Figure 6, different lipophilic parameters are grouped in two clusters. During the CA, a highly manifested extraction of alternative lipophilic parameters, m, into one cluster occurred. The second cluster, which can be divided into two subclusters, contains all the mathematically determined lipophilic parameters, log P, and chromatographic retention constants obtained in both used organic modifier. This distribution of lipophilicity parameters suggests that the retention constant R_M^0 shows a much greater similarity to the log P than chromatographic parameter m at given conditions.

What is important to emphasize is that one subcluster involves lipophilic parameters that were obtained experimentally, R_M^0 obtained in both used organic modifiers and some lipophilic parameters: mi log P, AC log P and "kowwin". The grouping of the studied lipophilic parameters in this way suggests that the experimental values are in better agreement with the standard measure of lipophilicity with which they formed the same cluster.

A very similar distribution of analyzed lipophilic parameters as in the case of CA was obtained by using PCA (Figure 7). All mathematically determined log P values and R_M^0, obtained from both used solvents, were grouped together, which once again proves their similarity. The m values measured in both modifiers appeared as an outlier, which can be interpreted as them having very little in common with standard measures of lipophilicity for the investigated phenylacetamide derivatives at the given conditions.

Conclusion

Lipophilic parameters of newly synthesized *N*-substituted-2-phenylacetamide derivatives were determined in two ways:

experimentally by using RPTLC with different organic modifiers (methanol and acetonitrile) and computationally from structural formula by applying different mathematical methods under the relevant software package. The obtained chromatographic results indicate that the retention of the investigated compounds depends on the nature on the substituent on the nitrogen atom and on the selected organic solvent. The aim of the study was to establish dependence between the partition coefficient log *P*, as a standard measure of lipophilicity and experimentally obtained R_M^0, and the *m* values, as an alternative measure of lipophilicity, by applying classical linear regression analysis and multivariate methods, CA and PCA. All used methods gave approximately similar groupings of the studied lipophilic parameters. The obtained results confirm that the chromatographic retention parameter of the investigated *N*-substituted-2-phenylacetamide derivatives obtained by RPTLC at given conditions, in contrast to the parameter *m*, shows strong similarity to the standard measure of lipophilicity obtained by different mathematical methods. Hereby, two important things are demonstrated. First, the chromatographic retention constant, R_M^0, unlike the chromatographic parameter *m*, could be successfully used for describing the lipophilicity of investigated compounds. Second, it demonstrates that the used multivariate methods are able to reduce the dimensionality and create a connection between the numbers of data obtained in different ways.

Acknowledgments

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