Hindawi
Journal of Analytical Methods in Chemistry
Volume 2019, Article ID 8131235, 10 pages
https://doi.org/10.1155/2019/8131235

Research Article

The Application of CA and PCA to the Evaluation of Lipophilicity and Physicochemical Properties of Tetracyclic Diazaphenothiazine Derivatives

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Received 5 July 2019; Accepted 17 September 2019; Published 20 October 2019

Academic Editor: Jaroon Jakmunee

The subject of the study was 11 new synthetized tetracyclic diazaphenothiazine derivatives. Using thin-layer chromatography in a reverse phase system (RP-TLC), their R_{M0} lipophilicity parameter was determined. The mobile phase was composed of 0.2 M Tris buffer (pH = 7.4) and acetone (POCH S.A., Gliwice, Poland) in different concentrations. Using computer programs, based on different computational algorithms, theoretical values of lipophilicity (AClogP, ALOGP, ALOGPs, miLogP, MLOGP, XLOGP2, and XLOGP3) as well as molecular descriptors (molecular weight, volume of a molecule, dipole moment, polar surface, and energy of HOMO orbitals and LUMO orbitals) and parameters of biological activity: human intestinal absorption (HIA), plasma protein binding (PPB), and blood-brain barrier (BBB), were determined. The correlations between the experimental values of lipophilicity and theoretically calculated lipophilic values and also between experimental values of lipophilicity and values of physicochemical or biological properties were assessed. A certain relationship between structure and lipophilicity was found. On the other hand, the relationships between R_{M0} and physicochemical or biological properties were not statistically significant and therefore unusable. For all analysed values, an analysis of similarities and principal component analyses were also made. The obtained dendrograms for the analysis of lipophilicity and physicochemical and biological properties indicate the relationship between experimental values of lipophilicity and structure in the case of theoretical lipophilicity values only. PCA, on the other hand, showed that ALOGP, MLOGP, miLogP, and BBB and molar volume have the largest share in the description of the entire system. Distribution of compounds on the area of factors also indicates the connections between them related to their structure.

1. Introduction

Designing new compounds which could find the application as drugs (or their precursors) is an expensive and time-consuming process. There is ongoing research concerning a new solution that could limit the number of necessary syntheses and at the same time obtain the products with optimal physicochemical properties correlated with biological activity [1]. QSAR (quantitative structure-activity relationship) is the leading method used in obtaining new drugs. It describes the quantitative relationship between the necessary biological activity of the compound and its chemical structure [2, 3]. To define the differences between a series of similar substances, the QSAR descriptors are used. They are physicochemical parameters that are an interpretation of the biological properties of the compounds investigated [4]. One of the most often used parameters in QSAR analysis is lipophilicity. It can be connected with all

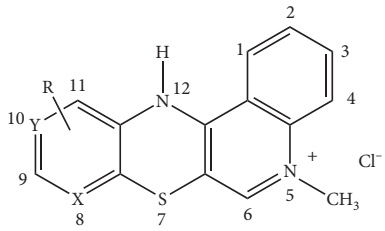
drug interaction phases in the body, i.e., pharmaceutical, pharmacokinetic, and pharmacodynamic phases [5]. In the pharmaceutical phase, it affects the form of the drug, the way it is administered, and the release in the body. Also, the processes of absorption and distribution of biologically active substances depend significantly on lipophilicity, which is the main factor determining the bioavailability of the drug, and therefore its solubility in bodily fluids and the ease with which it is transported through biological membranes. Moreover, the lipophilic properties of the drug substance affect the way it interacts with the target receptor, and thus the pharmacological effect [6]. Lipophilicity determines the affinity of the molecule to the organic phase. It is expressed as the partition of substances in a two-phase system: liquid-liquid or liquid-solid; most often, lipophilicity is described by partition processes between polar (organic) and nonpolar (water) phases [7]. The traditional method of its determination is extraction in the octanol-water system [8, 9]. Currently, the chromatographic methods in which the test substance undergoes partition in a dynamic system composed of the mobile and stationary phases are of great importance in the determination of lipophilicity parameters [10, 11]. This method precisely and simultaneously determines many test substances, whose distribution coefficient can be in a wide range. In thin-layer chromatography and high-performance liquid chromatography, octadecylsilanized silica gel (RP-18) is used as the stationary phase, which to some extent imitates the structure of long-chain fatty acids in biological membranes. The mobile phase is usually the water-organic solution [11–13]. One of the groups of the drugs that are important from the point of view of medicinal chemistry is phenothiazine neuroleptics, known since the 1950s. Initially, these compounds were used in psychiatry as antipsychotics [14, 15]. The basic structural unit of neuroleptic phenothiazine is a tricyclic system in which two benzene rings are connected by a nitrogen and sulfur atom to form a 1,4-thiazine ring, with an attached alkyl chain in the N-10 position [16]. It is a highly lipophilic system, which is related to the strong affinity of the phenothiazine molecule to the lipid bilayer of cell membranes of neurons and other lipid-rich tissues. This property of phenothiazines allows them to penetrate the blood-brain barrier, which determines the mechanism of neuroleptic action of these compounds [17, 18]. Modification of the basic tricyclic phenothiazine structure can be accomplished by introducing new pharmacophore substituents into the thiazine ring or benzene rings and by replacing the benzene rings with heterocyclic systems, e.g., pyridine, quinoline, or pyrimidine. These types of structure transformations can lead to new compounds with a different direction and impact force [19, 20]. Literature reports indicate a number of promising types of biological activity of classical phenothiazines and their new derivatives, including antibacterial [21–24], antifungal [25], antiprional [26], antiprotozoal [27, 28], and antiviral [29, 30]. In addition, the antitumor properties of phenothiazines and their ability to modify multidrug resistance of certain tumor cell lines have been described [31, 32]. A new group of phenothiazine derivatives is tetracyclic quinobenzothiazine derivatives, which were obtained by replacing one of the benzene rings with a quinoline ring [33, 34]. For a number of derivatives of this type, the lipophilicity parameters R_{M0} and $\log P_{TLC}$ (theoretical and experimental) were determined [35]. Moreover, their interesting anticancer properties have been described in relation to human tumor cells of the MDA-MB-231 line (breast cancer), SNB-19 (brain glioma), and C-32 (cutaneous melanoma) [36]. Using the conversion of the benzene ring in the quinobenzothiazine system to the pyridine ring, a number of new compounds have been synthetized containing different substituents at various positions of the pyridine ring. The aim of this work is to determine the lipophilicity parameters (R_{M0}, $\log P_{TLC}$, and $\log P_{calc}$) of 15 new pyridoquinothiazine salts by thin-layer chromatography in the reverse phase of RP-TLC and using computational methods and correlating them with each other. In addition, the R_{M0} lipophilicity parameter will be correlated with other molecular descriptors, and with theoretical values of biological activity.

2. Materials and Methods

2.1. Chemicals. The series of pyridoquinothiazine derivatives, described by symbols **1**–**11**, were investigated. The structures of compounds are shown in Figure 1.

Derivatives **1**–**11** were obtained through synthesis of thiochininantrenodiic chloride with a series of substituted aminopyridine derivatives. The methodology was described in our previous work [37]. The basic structure of the unsubstituted pyridoquinothiazine salt containing the nitrogen atom at position 8 of the tetracyclic system was modified by introducing various types of electron-withdrawing substituents and electron donors (Br, Cl, F, I, CH_3, and OCH_3) in positions 9, 10, and 11 of the pyridine ring. The previously unsubstituted **11** derivative has a nitrogen atom at position 10 of the pyridoquinothiazine system. The compounds have an additional methyl group on the pyridine or imine nitrogen atom. The structure of all compounds was confirmed by ESI-HRMS spectrometry and ^1H, ^{13}C NMR spectroscopy, using two-dimensional techniques HSQC, HMBC, NOESY, and COSY.

2.2. Thin-Layer Chromatography. The lipophilicity parameters were experimentally determined by reverse phase thin-layer chromatography (RP-TLC). Chromatograms were prepared on RP-18F_{254s} plates (1.05559.0001, Merck, Germany) precoated with nonpolar silicone oil. Plates were developed in glass chromatography chambers (Chromdes, Lublin, Poland) previously saturated with the vapour of the mobile phase. Solutions of pyridoquinothiazine salts **1**–**11** were prepared by dissolving 1 mg of particular compound in 2 ml of ethanol (POCH S.A., Gliwice, Poland). The solution was spotted into chromatographic plates in the amount of 2 μL by use of microcapillary. The mobile phase was composed of 0.2 M Tris buffer (pH = 7.4) and acetone (POCH S.A., Gliwice, Poland) in different concentrations, i.e., 50%, 60%, 70%, 80%, and 90%. The chromatograms were visualised in UV light (λ = 254 nm). Determination of the R_F coefficient was carried twice for all compounds and in all acetone concentrations used. The final value of R_F is the



	R	X	Y
1	H	N	CH
2	9-Br	N	CH
3	10-Br	N	CH
4	9-Cl	N	CH
5	9-F	N	CH
6	9-I	N	CH
7	9-Cl 11-CH_3	N	CH
8	9-F 11-CH_3	N	CH
9	11-CH_3	N	CH
10	9-OCH_3	N	CH
11	H	CH	N

Figure 1: General structure of pyridoquinothiazine derivatives investigated (**1**–**11**).

mean of the two measurements. The obtained R_F coefficient was used to calculate the value of the R_M parameter, according to the following equation:

$$R_M = \log\left[\left(\frac{1}{R_f}\right) - 1\right]. \quad (1)$$

By extrapolating R_M values to zero acetone concentration, a relative R_{M0} lipophilicity parameter was obtained, according to the following equation:

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

where b is the slope and C is the concentration of acetone in mobile phase.

2.3. Computational Programs. For pyridoquinothiazine derivatives **1**–**11**, the theoretical values of the $\log P_{calc}$ parameter were determined using computer modules whose calculations are based on atoms, molecular fragments, and molecular properties (AClogP, ALOGP, ALOGPs, miLogP, MLOGP, XLOGP2, and XLOGP3) [38, 39]. The calculation methods used for obtaining the $\log P$ values were described earlier in our work concerning another group of new synthetized compounds [40]. Molecular descriptors (molecular weight, volume of a molecule, dipole moment, polar surface, and energy of HOMO orbitals and LUMO orbitals) were calculated using the DFT (density functional theory) method. A hybrid B3LYP function was used. Parameters of biological activity: human intestinal absorption (HIA), plasma protein binding (PPB), and blood-brain barrier (BBB), were determined using virtual computational bases [41].

2.4. Correlation, Cluster Analysis, and Principal Component Analysis. Based on the values of experimental lipophilicity (R_{M0}, $\log P_{TLC}$) and theoretical parameters ($\log P_{calc}$), as well as the determined values of molecular descriptors and parameters of the predicted biological activity, correlation analysis, cluster analysis (CA), and principal component analysis (PCA) were performed. All data used for CA and PCA were standardised. The cluster analysis was based on the Euclidean distance, a single linkage method. The PCA analysis was based on the correlation matrix, using the Kaiser criterion and scree plot. The entire analysis was carried out using the Statistica 13.1 software.

3. Results and Discussion

The values of $\log P_{TLC}$, for compounds investigated, were calculated on the basis of known values of R_{M0}, which were obtained by chromatographic analysis. In order to obtain the values of the $\log P_{TLC}$ parameter for the tested derivatives **1**–**11**, first, the analysis of standard substances with the well-known lipophilicity value ($\log P_{lit}$) was performed. The analysis was carried out under the same chromatographic conditions as for the tested substances. The reference compounds analysed were acetanilide **I**, p-cresol **II**, p-bromoacetophenone **III**, benzophenone **IV**, and anthracene **V**. Values of lipophilicity parameters of reference substances: literature ($\log P_{lit}$), experimental (R_{M0}), and $\log P_{TLC}$, are presented in Table 1. Differences between the value of $\log P_{lit}$, and $\log P_{TLC}$ for the standards did not exceed ±0.190 and were below ±0.150 for three compounds.

Using the $\log P_{lit}$ relation from the experimentally determined R_{M0} parameters for the standards, a calibration curve was made. The linear function describing the calibration curve led to the formulation of the equation according to which $\log P_{TLC}$ was determined for the tested derivatives:

$$\log P_{TLC} = 1.4664R_{M0} + 0.1043 \quad (r = 0.993, SD = 0.162, F = 230.9). \quad (3)$$

The R_{M0} parameter values and $\log P_{TLC}$ values for derivatives **1**–**11** are shown in Table 2.

The $\log P_{TLC}$ parameters for the tested derivatives were within the range of 2.92–5.78. The collected results indicate the dependence of lipophilicity on the structure of the molecule as well as the presence and type of substituents at various positions of the pyridoquinothiazine system. Compounds with only an additional alkyl group: 11-CH3 (**9**, $\log P_{TLC}$: 4.19) and 9-OCH_3 (**10**, $\log P_{TLC}$: 4.22), were characterised by lower values of lipophilicity. The introduction of the halogen atom in the system containing the −11 methyl group resulted in a significant increase in the $\log P_{TLC}$ parameter in relation to the initial compound, as observed for the derivatives **7** (9-Cl, $\log P_{TLC}$: 5.72) and **8** (9-F, $\log P_{TCL}$: 5.78). A significant reduction in lipophilicity was noted for the unsubstituted derivative **1** ($\log P_{TLC}$: 3.53) and for the isomeric derivative **11** ($\log P_{TLC}$: 2.92) in which the nitrogen atom of the pyridine ring is located in position 10 of the diazaphenothiazine system.

One of the stages of the research was the determination of the $\log P$ parameter using computer methods. Depending on the mathematical module of the program used, the obtained values of the $\log P_{calc}$ parameter occurred in a very wide range from −1.16 to 4.70 (Table 3).

It was not possible to obtain a difference fewer than 0.5 units between the parameters calculated $\log P_{calc}$ and experimental $\log P_{TLC}$ for all tested compounds in any of the

Table 1: Values of $\log P_{lit}$ parameters, experimentally determined R_{M0}, and $\log P_{TLC}$ for reference substances **I**–**V**.

Lipophilicity	Standard substances				
	I [42]	II [43]	III [44]	IV [42]	V [44]
$\log P_{lit}$	1.2100	1.9700	2.4300	3.1800	4.4500
R_{M0}	0.6800	1.2930	1.6260	2.2240	2.8500
$-b$	0.0119	0.0196	0.0207	0.0280	0.0335
r	0.9908	0.9953	0.9945	0.9985	0.9989
SD	0.0296	0.0346	0.0398	0.0278	0.0282
$\log P_{TLC}$	1.0670	2.0380	2.5090	3.3630	4.2610

b: slope; r: correlation coefficient; SD: standard error.

Table 2: The values of the R_{M0} parameter obtained on the basis of the equation $R_M = R_{M0} + (b)C$ and the value of $\log P_{TLC}$ for the **1**–**11** derivatives.

No	R_{M0}	$-b$	r	SD	$\log P_{TLC}$
1	2.3379	0.0278	0.9957	0.0470	3.53
2	3.2293	0.0391	0.9924	0.0882	4.83
3	3.0699	0.0361	0.9900	0.0935	4.61
4	3.1016	0.0377	0.9907	0.0943	4.65
5	2.8262	0.0343	0.9946	0.0648	4.25
6	3.4373	0.0410	0.9900	0.1062	5.14
7	3.8299	0.0450	0.9934	0.0943	5.72
8	3.8738	0.0463	0.9913	0.1122	5.78
9	2.7871	0.0333	0.9906	0.0850	4.19
10	2.8087	0.0325	0.9941	0.0649	4.22
11	1.9173	0.0241	0.9961	0.0386	2.92

programs used. Differing values of results stem from the method of counting lipophilicity. The computer programs calculating the lipophilicity parameters are based on the structure of neutral molecules. It does not take into account the influence of conformation, tautomerisation, ionisation, changes in electron density, or the formation of hydrogen bondsor ion pairs through compounds investigated. Moreover, literature reports indicate a significant influence of changes in the thiazine ring conformation and changes in electron density at individual nitrogen atoms on the lipophilicity of quinobenzothiazine tetradenic derivatives [45].

In order to determine the relationship between experimental and theoretically calculated parameters, a correlation analysis was performed for the R_{M0} and $\log P_{calc}$ parameters. In the studied group of derivatives, high and very high values of correlation coefficients were obtained for all computer modules used ($r = 0.7555$–0.9604, $p < 0.05$). Also, the $\log P$ values analysed correlated well with each other. With respect to these results, it can be stated that there is a relationship between the structure and the lipophilicity of the compounds tested. The correlation equations obtained are presented in Table 4.

As part of the work, attempts were made to correlate the lipophilicity parameters of derivatives **1**–**11** with the values of physicochemical properties such as the molar mass, volume of the molecule, dipole moment, polar surface, and HOMO-LUMO gap (Table 5).

In the group of analysed compounds, the best correlation of the R_{M0} parameter was obtained for the relation with the volume of the molecule ($r = 0.8225$). Statistically significant correlations were also obtained for relations with dipole moment ($r = 0.6626$) and with gap energy ($r = 0.6496$). Correlations with other physicochemical properties were statistically insignificant.

As mentioned earlier, lipophilicity has a significant impact on the behaviour of biologically active compounds in the human body, including their absorption when taken orally, binding to proteins in the bloodstream and penetration of the blood-brain barrier or blood-placenta barrier. Therefore, it seemed appropriate to carry out a correlation study of the relative lipophilicity parameter R_{M0} with the calculated HIA parameter (human intestinal absorption), the PPB parameter (plasma protein binding), and the BBB parameter (blood-brain barrier–blood-brain penetration factor). The values of biological activity coefficients are presented in Table 6.

The results suggest very good oral bioavailability of all test compounds **1**–**11** (HIA > 96%) (Table 6). The blood-brain barrier penetration ratio in both groups is also high for newly obtained salts and is in the range of 89.51–97.28%, which classifies them as potential neuroleptic drugs. The highest BBB parameter value was calculated for derivative **10** containing a 9-methoxy group (BBB = 97.28%). The degree of protein binding is more diverse and depends on the structure of the compound being analysed. For salts **1**–**11,** it ranged from 20.32 to 88.16%. The theoretical value of this coefficient affects the concentration of the free fraction of the compound in the bloodstream and thus its biological activity: the higher the PPB, the lower the biological activity. The lowest values of this parameter were noted for the isomeric derivative **11** (PPB = 20.32%). The correlation of the R_{M0} parameter with the calculated parameters of biological activity resulted in different regression coefficients. In the group of analyzed compounds, only for the relationship with the PPB parameter, a high positive statistically significant correlation was obtained ($r = 0.7284$). The remaining results indicate that there is no significant relationship between the analysed features; hence, correlation equations describing such relationships cannot be used to determine the R_{M0} parameter. Taking into account the above results, Table 7 presents correlation equations describing the relationships between lipophilicity values and physicochemical or biological parameters, but only those which are statistically significant.

For an additional description of the analysed compounds, the similarities were analysed, taking into account the lipophilicity values separately (experimental and theoretically calculated), values of physicochemical properties, and HIA, PPB, and BBB values. The obtained results are presented in the form of dendrograms. The first dendrogram (Figure 2) presents the similarity between analysed compounds based on their values of lipophilicity.

As a result of the analysis of similarities of lipophilicity values for the analysed compounds, several clusters are observed. Compounds 7 and 8 contain a hydrophobic methyl group at position 11. Moreover, they contain also the highly ellectronically accepting atoms at position 9, F in the case of compound 8 and Cl in the case of compound 7.

Table 3: Values of lipophilicity parameters of 5-methyl-12 (*H*)-quin [3,4-*b*] pyrido [2,3-*e*] [1, 4] thiazine salts 1–11 obtained using computer methods.

No.	AClogP	ALOGP	ALOGPs	miLogP	MLOGP	XLOGP2	XLOGP3
1	2.74	3.35	−0.90	−0.13	1.38	2.69	3.27
2	3.35	4.33	−0.12	1.02	2.02	3.58	4.30
3	3.44	4.09	−0.30	0.83	2.02	3.49	3.96
4	3.44	4.22	−0.15	0.89	1.90	3.40	4.23
5	3.28	3.93	−0.42	0.38	1.78	2.94	3.71
6	3.76	4.45	−0.13	1.30	2.14	3.85	3.96
7	3.76	4.70	0.01	1.27	2.14	3.63	4.60
8	3.59	4.42	−0.28	0.75	2.02	3.17	4.07
9	3.06	3.83	−0.75	0.25	1.63	2.92	3.64
10	3.11	3.87	−0.58	0.27	1.91	3.34	3.58
11	2.65	2.81	−1.16	−0.33	1.38	2.61	2.94

Table 4: Correlation equations for relationships between R_{M0} and lipophilicity values theoretically calculated for compounds **1**–**11**, $p < 0.05$.

	Correlation equation	r
AClogP	$R_{M0} = 1.4803 \log P_{calc} - 1.8488$	0.9382
ALOGP	$R_{M0} = 1.0402 \log P_{calc} - 1.1408$	0.9604
ALOGPs	$R_{M0} = 1.4052 \log P_{calc} + 3.6305$	0.8901
miLogP	$R_{M0} = 0.9497 \log P_{calc} + 2.4587$	0.8811
MLOGP	$R_{M0} = 1.9049 \log P_{calc} - 0.499$	0.8944
XLOGP2	$R_{M0} = 1.0943 \log P_{calc} - 0.5237$	0.7555
XLOGP3	$R_{M0} = 1.0971 \log P_{calc} - 1.195$	0.8950

Table 5: Values of physicochemical properties for derivatives **1**–**11**.

No.	M_M	μ (D)	V_M (Å^3)	PSA (Å^2)	HOMO (eV)	LUMO (eV)	Gap (eV)
1	266.342	12.1387	236.8	32.57	−0.323134	−0.220941	−0.10219
2	345.236	12.355	254.69	32.57	−0.324275	−0.224889	−0.09939
3	345.236	13.4737	254.69	32.57	−0.325208	−0.225792	−0.09942
4	300.785	12.4105	250.34	32.57	−0.324502	−0.223992	−0.10051
5	284.330	12.4232	241.73	32.57	−0.327587	−0.225137	−0.10245
6	392.236	13.2687	260.79	32.57	−0.320785	−0.225700	−0.09509
7	314.811	13.7753	266.9	32.57	−0.32108	−0.222058	−0.09902
8	298.357	13.8931	258.29	32.57	−0.324334	−0.222905	−0.10143
9	280.367	13.6158	256.36	32.57	−0.319921	−0.21898	−0.10094
10	296.366	14.8075	262.35	41.80	−0.312113	−0.213951	−0.09816
11	266.339	9.4344	230.61	32.57	−0.331136	−0.223702	−0.10743

Table 6: Values of factors of biological activity (HIA, PPB, and BBB) for compounds **1**–**11**.

No.	HIA	PPB	BBB
1	96.36	58.00	92.86
2	96.96	88.25	90.18
3	96.96	69.39	90.18
4	96.75	78.07	90.93
5	96.36	54.44	91.51
6	97.23	89.16	89.51
7	96.82	76.16	90.76
8	96.46	64.04	91.44
9	96.45	63.65	92.76
10	96.35	45.74	97.28
11	96.35	20.32	94.38

Compounds **5**, **9,** and **10** contain electronegative F atom at position 9 and a methyl or methoxy group. The compounds **2**, **3,** and **4** contain in their structure, in the 9 or 10 position, a Cl or Br atom. The last cluster is formed by compounds **1** and **11**. They do not contain any additional substituents in the pyridine ring. In this case, it can be assumed that the change in the position of the N atom from position 8 to 9 has no significant effect on lipophilicity.

Table 7: Correlation of the R_{M0} parameter with physicochemical properties (dipole moment μ, volume of molecule V_M, and HOMO-LUMO energy difference (gap)) and PPB for derivatives **1**–**11**, $p < 0.05$.

Structural descriptor	Correlation equation	r
μ	$R_{M0} = 0.2764\mu - 0.5379$	0.6626
V_M	$R_{M0} = 00426V_M - 7.7315$	0.8225
Gap	$R_{M0} = 123.3884\text{gap} + 15.4264$	0.6495
PPB	$R_{M0} = 0.0214\text{PPB}\% + 1.6425$	0.7284

The second similarity analysis was done for values of physicochemical properties for all compounds investigated (Figure 3).

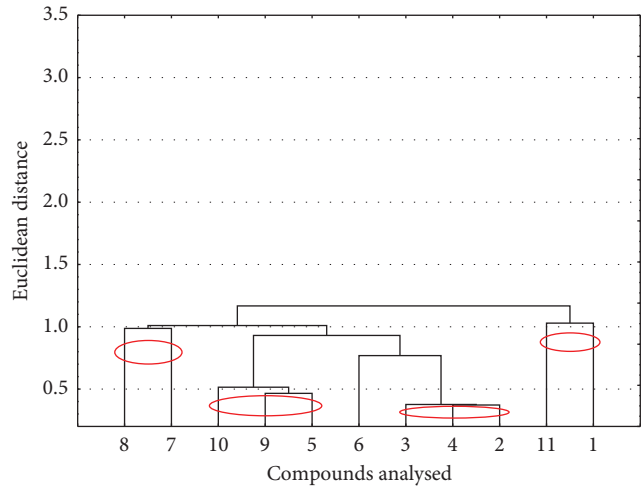


Figure 2: Similarity analysis for compounds investigated based on their values of lipophilicity.

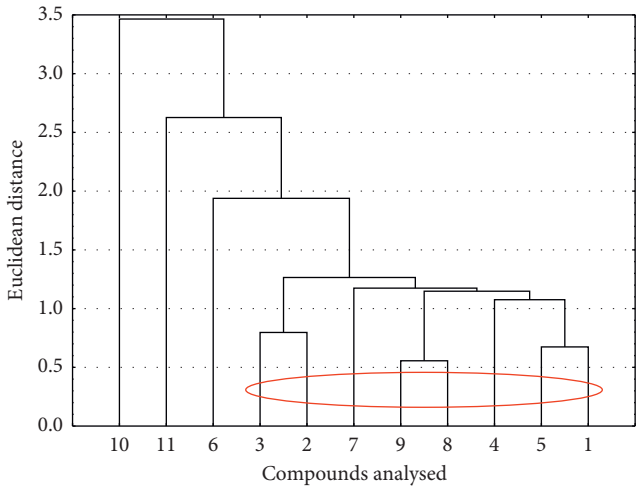


Figure 3: Similarity analysis for compounds investigated based on their values of physicochemical properties.

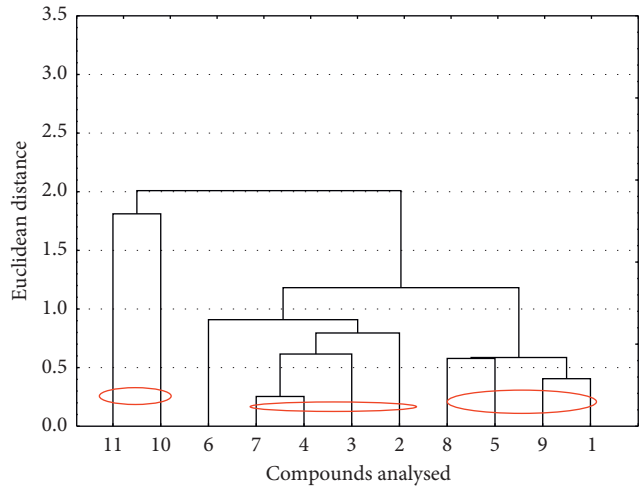


Figure 4: Similarity analysis for compounds investigated based on their values of biological properties.

Based on the data analysis of the physicochemical properties, there is one large cluster containing all of the compounds tested, except for 6, 10, and 11. This may be due to the fact that compound **6** has an iodine atom with a large volume in its structure, whereas compound **10**—methoxy group—and compound **11** have a nitrogen atom at position 10 of the pyridine ring. The remaining compounds show great similarity taking into consideration the physicochemical properties.

The next similarity analysis was done for values of biological properties (HIA, PPB, and BBB) for all compounds investigated (Figure 4).

Several clusters were obtained as a result of the analysis. Compounds **2**, **3**, **4,** and **7** contain a Cl or Br atom in positions 9 and 10. In addition, it turned out that compounds **10** and **11** are similar in biological properties, while compounds **1**, **5**, **8,** and **9** are the most similar and form a separate cluster. It should also be emphasised that the compounds investigated show the largest similarity when only the lipophilicity (theoretical and experimental) values were taken into account. For them, the Euclidean distance was the smallest.

For a better description of the obtained experimental and theoretical data for the tested compounds, the principal components analysis (PCA) was also used. Eigenvalues were extracted based on all data. Three main components were selected using the Kaiser criterion, the value of which exceeds 1. These selected components in 94.13% describe the variability of the system. However, when analysing the scree plot presented in Figure 5, it can be concluded that the main components should be 4. Then, the variability of the system would be described in almost 97%.

Figure 6 presents a view of variables on the area of factors, showing the share of individual variables in the main components. The longest vectors showing the largest share are characterised by the following variables: ALOGP, MLOGP, miLogP, and BBB and molar volume. Most of these variables are closely related to the structure of the

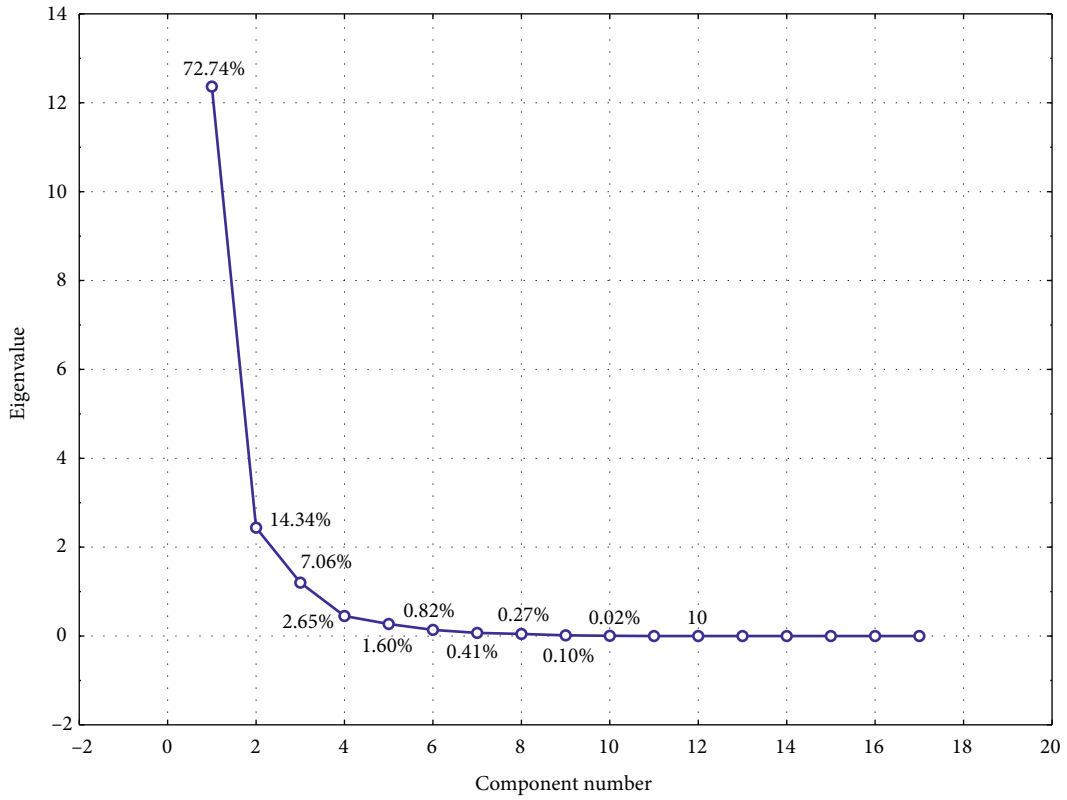


Figure 5: Scree plot for experimental and theoretical data analysed.

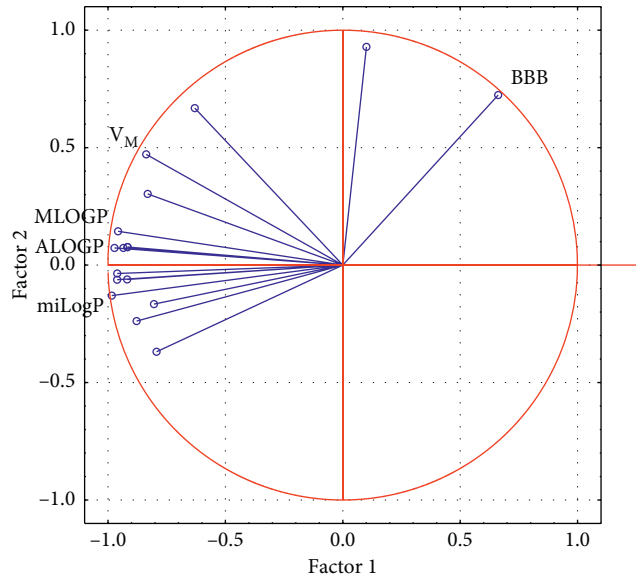


Figure 6: Projection of variables on the area of factors based on the first two eigenvalues.

analysed compounds, which was confirmed by previous analyses.

Through PCA analysis, the distribution of cases (compounds) on the area of factors can be presented (Figure 7).

It is clearly visible that the analysed compounds practically form one group, with the exception of compound **10**. It can most certainly be related to the structure of the compound, as compound **10** is the only one with a methoxy group in position 9 in its structure. The location of compounds **1** and **11** in one place may also indicate a connection with their structure because only they, from all tested compounds, have no substituents.

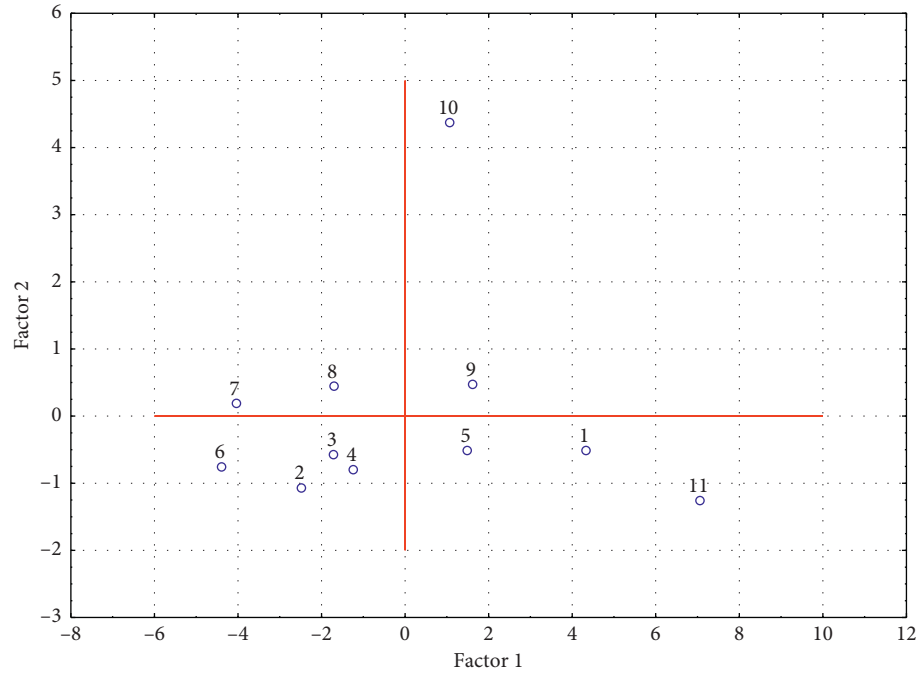


Figure 7: The distribution of cases (compounds) on the area of factors.

4. Conclusion

The subject of the study was new synthetized tetracyclic diazaphenothiazine derivatives. Using thin-layer chromatography in an inverse phase system (RP-TLC), the R_{M0} lipophilicity parameter for these was determined. Using computer programs, based on different computational algorithms, theoretical, mainly based on compound structure, lipophilicity values, as well as physicochemical and biological properties were determined. It can be concluded, through analysis of the obtained correlations between the experimental values of lipophilicity and the theoretically calculated lipophilic values, that there is a certain relationship between structure and lipophilicity. The relationships between R_{M0} and ALOGP and AClogP values are characterised by high values of correlation coefficients, 0.9604 and 0.9382, respectively. On the other hand, relationships between R_{M0} and physicochemical or biological properties were not statistically significant and therefore unusable. For all analysed values, an analysis of similarities and principal component analyses were also made. The obtained dendrograms for the analysis of lipophilicity and physicochemical and biological properties indicate the relationship between experimental values of lipophilicity and structure, but only in the case of theoretical lipophilicity values. PCA, on the other hand, showed that ALOGP, MLOGP, miLogP, and BBB and molar volume have the largest share in the description of the entire system. The distribution of compounds on the area of factors also indicates the connections between them related to their structure.

Data Availability

All chromatographic data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

This research was financed by the Medical University of Silesia as part of statutory research (no. KNW-1-055/K/9/O).

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