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FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING**

**The study of lipophilicity for new thiazole and selenazol
derivatives with biological potential by reversed-phase
liquid chromatography**

SUMMARY

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CONTENTS

INTRODUCTION	3
1 ACTUAL STATE OF KNOWLEDGE	4
2 AIM AND OBJECTIVES	7
3 MATERIALS AND METHODS	8
4 RESULTS AND DISCUSSIONS	9
4.1 The study of lipophilicity for 8 new derivatives of <i>p</i> -toluensulfonyl - hidrazino-thiazole by thin-layer chromatography and high-performance reversed-phase liquid chromatography	10
4.2 The study of lipophilicity for 12 newly synthesized selenazol derivatives by thin -layer chromatography with reversed- phases	14
4.3 The study of lipophilicity for 17 newly synthesized selenazole derivatives by reversed-phases liquid chromatography	18
4.4 The study of lipophilicity for 14 new phenothiazin- hidrazino-thiazole derivatives by thin- layer chromatography method	21
5 CONCLUSIONS AND RECOMMENDATIONS	24
BIBLIOGRAPHY	27
LISTS OF THE PAPERS THAT WERE ACCEPTED FOR PUBLICATION	30

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SPSS1

INTRODUCTION

The biological activity of medicinal substances involves initial interaction of chemical, biochemical or physico-chemical type with the components of living matter molecules. Thus, bioactive compounds penetration through hydrophobic cellular membranes to reach the target organ, the biological activity, is expressed by lipophilicity. Because lipophilicity indirectly describes the process of absorption, distribution, metabolism and elimination, it is considered a very important parameter in monitoring the behavior of a chemical compound in the biological environment. In this respect, it is necessary to determine lipophilicity for all new compounds, as a completion to their properties. Among the experimental determination of lipophilicity techniques, chromatographic methods are considered safe and highly reproducible and accurate results are obtained even for series of compounds that are structurally different. The quality of results is due to the fact that, in order to estimate lipophilicity for a series of compounds, one can address several chromatographic techniques and the results can validate each other.

Currently, lipophilicity of compounds can be expressed by theoretical methods. Thus, based on the structure, by means of algorithms included in computer programs, a large number of theoretical descriptors can be obtained, that are to be correlated with the experimental results, validated by statistical analysis.

This research theme is the study of the lipophilicity of new derivatives of thiazole and selenazol by reversed-phase liquid chromatography (thin-layer chromatography and column chromatography) and by computational methods.

The general objectives identified are: description of the behavior of chemical species in a given context, conducting investigations to detect properties and relationships, draw conclusions using information from graphs, experimental data, documentary sources that will correspond to the assumptions made. Some results have been accepted for publication in specialized magazines abroad and in our country.

1 ACTUAL STATE OF KNOWLEDGE

According to IUPAC, lipophilicity is a physico-chemical property that describes an equilibrium of distribution of the molecules dissolved in water and an immiscible organic solvent, favoring the latter and it is correlated with bioaccumulation [1]. In this respect, the distribution coefficients between a non-polar organic phase and water are measurements that express the lipophilic character of a compound. A small or a negative distribution coefficient shows affinity of the compound for water, while a high value of the distribution coefficient expresses to what extent the analyzed compound is distributed mainly in non-aqueous phase compared to the aqueous one. High value of distribution coefficient is directly associated with high lipophilicity of the compounds. To estimate lipophilicity of the compounds can be used both theoretical methods and experimental methods. Theoretical estimation methods of lipophilicity indices are based on structural features, as a consequence of computational chemistry development. This category includes calculation methods based on structural features and calculation methods that take into account the properties of the compounds analyzed [2-5].

The method of separating funnel, as a method for directly determining the lipophilicity, respectively the chromatographic methods, considered as indirect methods of determining lipophilicity, allow the experimental determination of lipophilicity. Among the methods of chromatography, liquid chromatography is preferred in estimating lipophilicity because of the similarity of distribution of the solute in a chromatographic system, which requires a liquid mobile phase and stationary phase, and the distribution of the solute in a dual liquid environment. Some of the advantages of chromatographic methods in comparison to the method of separating funnel are: a greater speed of determination, better reproducibility, it requires small amounts of compound and the analyzed compound should not be very pure.

In thin-layer chromatography, the chromatographic descriptors of lipophilicity are influenced by: the specific area surface, density of active centers per unit surface area, energy of the intermolecular interactions occurring between the active centers of sorbent type and a given molecule, and the chemical structure of the sorbent [6].

Chromatographic behavior of a compound is determined by the stationary phase. In this respect, the thin-layer chromatography uses to estimate lipophilicity the cellulose and silicagel as stationary phases. Also, many lipophilicity studies use as stationary phase silicagel impregnated with various oils eg.paraffin oil, silicone oil or C8 or C18 hydrocarbon chain derivatives [7-9]. The most known lipophilicity indicators used in thin-layer chromatography are based on the retention factors (R_f) and are calculated by the relationship:

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

where R_M is a parameter defined in a similar way as the capacity factor (k), a parameter common for the column liquid chromatography, too [10], and R_f represents the retention factor calculated from the ratio of the migration distance of the compound and the solvent front migration distance. From the linear relationship established between R_M values and the concentration of the organic modifier from the mobile phase, can be calculated an extrapolated value (R_{M0}) even for more lipophilic compounds for which the low concentration measurements of organic modifier in the mobile phase are not possible. The validity of the extrapolation technique was supported by the fact that very good correlations were obtained between the R_{M0} values and the log p values into the octanol – water system [11]. It was also confirmed that there is a close correlation between the R_{M0} values and slope (S), so that in many cases, the slope is associated with a specific hydrophobicity of the surface, being considered an alternative measure of lipophilicity [12.13]. Among the factors affecting measurement of the lipophilicity by reversed-phase liquid chromatography column (RP-HPLC, reversed-phase high performance liquid chromatography) are stationary phase, mobile phase composition, organic modifier etc. The most common stationary phase used in the studies of the lipophilicity by RP-HPLC, contains chains of C8 and C18 carbon atoms fixed on a silica gel base, as this system is closest to the classical octanol-water system that is implemented in the separating funnel. Beside silicagel-based stationary phases, there were investigated other stationary phases in order to find correlations between retention factor and lipophilicity [14-19].

Another factor that must be taken in consideration when using reversed-phase liquid chromatography method for the determination of the partition coefficients is the choice of the mobile phase. Factors which depend on this choice are: organic modifier concentration, the concentration or the concentration field of the organic modifier, the need to use a buffer in the mobile phase, the need to use the stationary phase modifiers and, not least, the fact that it will be used the capacity factor extrapolated to 0% organic modifier ($\log K_{ow}$) or the capacity factor for a certain percentage of organic solvent ($\log k$) [20-22]. Organic modifiers commonly used in RP-HPLC are methanol, acetonitrile and tetrahydrofuran although it was proved that methanol has the best ability to solvate and it is adsorbed on stationary phases of chemically modified silica gel [23, 24].

There are many studies indicating the use of $\log K_{ow}$ for studies on lipophilicity. Many of these use binary mobile phases with methanol-water or methanol-aqueous buffer, with the $\log K_{ow}$ derived from extrapolations of methanol content [25-29]. Because of the dependence between the $\log k$ values and the mobile phase composition, it was tried to find a different chromatographic parameter that is less dependent on working conditions and can be universally used. In this respect, it was shown that the slope S may be an indicator of lipophilicity [30]. Another parameter for the estimation of lipophilicity in RP-HPLC is the chromatographic hydrophobicity index (ϕ_0) [31].

Models based on relationships between structure and biological activity have been used extensively in recent years, in researches on the synthesis of compounds with directed properties, modeling and optimization of various properties of newly synthesized compounds, toxicity study and, not least, the actions of decision on human health and environmental protection [32]. Despite significant progress on experimental and theoretical methods and computer processing the data, models based on structural – activity relations depend, to a large extent, on the size and diversity of the set of compounds studied, the experimental measurement errors, the diversity of sizes measured or calculated descriptors, but especially the mathematical algorithm that was used. The performance and utility of a model depends primarily on the strength of prediction of the properties of the unknown compounds and their biological activity [33-35].

These new lines of research have proved to be the cheapest methods, but the most efficient, too, in the design and testing of potentially active compounds (antioxidants, drugs, etc.).

2 AIM AND OBJECTIVES

For three series of new compounds with biological action, special attention was paid to the study of lipophilicity, property that plays an important role in the processes of absorption, distribution, metabolism and elimination.

The purpose of this study is to express the experimental and theoretical behaviour of some lipophilicity indicators and the evaluation of the relationship between the activity and the biological structure through computational methods.

Following the current state of knowledge, we have identified the following specific objectives: to predict lipophilicity for the selenazol and thiazole compounds type considered in this study, to establish interaction with the cell membrane based on the lipophilicity property, to set the parameters with the utmost influence of lipophilicity for the compounds in the two classes.

3 MATERIALS AND METHODS

The compounds which represent the research material were synthesized by a group of chemists from the University of Medicine and Pharmacy "Iuliu Hațieganu" in Cluj-Napoca. There are three types of compounds from the thiazole and selenazol class. Their structure was confirmed by analysis of IR spectra, MS and H-NMR [36].

RP-TLC: Experimental Apparatus and materials

Analyzed substances were purchased from the Department of Organic Chemistry, University of Medicine and Pharmacy "Iuliu Hațieganu" in Cluj-Napoca, where practically they were synthesized. Methanol used in the mobile phase composition was of high purity, being purchased through the company marketing for Merck (Darmstadt, Germany). Chromatographic behavior of the 12 compounds was studied using silica gel plates of RP-18WF254s type (10x10 cm, Merck). Solutions of the analyzed compounds were prepared in methanol (1mg/ml). The plates were applied 2 ml spots on the starting line with a Hamilton syringe. Before developing stage, rooms were saturated with mobile phase (methanol / water) for 30 minutes. All development was done at room temperature (22 ± 20 C degrees). Distance between home and migration front eluent was 8 cm in all cases. Concentrations of mobile phase (methanol / water) were used between 50 and 70% \pm 5%. After development, plates were dried at room temperature and then examined under UV light at a wavelength $\lambda = 254$ nm. The lamp used for viewing spots was type VL-6.C Vilberlourmat, of French production.

RP-HPLC experimental apparatus, material

In order to be prepared for chromatographic analysis, samples were prepared as follows: in an Eppendorf tube, 1 mL methanol was added to about 1 mg sample. The tube was subjected to ultrasound for 10 seconds and then centrifuged for 2 minutes at 12,000 rpm. Supernatant was transferred into a glass (bottle) sampling. Chromatographic

experiment was conducted using a HPLC (Agilent Technologies Series 1100), coupled with a UV detector (type G1314A) at 210 nm.

The column used was Zorbax SB-C18 type (100 x 3.0 mm, 3.5 mm). Mobile phase was prepared in methanol and water in different proportions, between 45-90% with increases of 5%. Before injection, the sample was dissolved in methanol and then diluted with water. Injected sample volume was 10 ml. Retention times were measured at 25 C degrees with UV detector. Dead time corresponding to the peak was solvent (C18) = 0.45 min. In the case of C18 column, the debit to which measurements were made was 1mL/min. All solvents used were obtained from Merck company, being of high purity.

4 RESULTS AND DISCUSSIONS

To achieve the purpose, an experimental plan was drawn up for all four experimental series of compounds analyzed. The specific objectives that make up the experimental plan are:

÷ separation of the compounds considered in the study by thin-layer chromatography and / or by column chromatography with reversed-phase;

÷ the extraction of the series of the lipophilicity indicators from the experimental data;

÷ modeling of the molecular structure and extraction of a series of theoretical indicators that characterize the structure;

÷ the analysis of interactions occurring at the cell membrane through the lipophilicity indices, respectively the lipophilicity estimators indices;

÷ to obtain the relationships between structure and activity, and on this basis to establish the parameters and / or structure indices mostly influencing the separation properties observed, and respectively, the estimated lipophilicity indicators.

4.1 The study of lipophilicity for 8 new derivatives of p-toluensulfonyl - hidrazino-thiazole by thin- layer chromatography and high-performance reversed-phase liquid chromatography

Because of the biological potential of the compounds with a thiazole function, expressed by antimicrobial, antitumor, and anti-inflammatory properties [37-39], a special attention is required to the physico-chemical properties that are directly correlated with biological activity. In this sense, the theme of this work is to study the lipophilicity of the p-toluensulfonile-hidrazino-thiazole compounds. The methods used for this purpose are liquid chromatography on thin-layer and on high performance column, with reversed-phase. Following the presented experimental plan, after the compounds which were considered in the study were separated by the two chromatographic methods, there were noted the retention factors (R_f) and retention times (t_R) (**Tables 3.3 and 3.4 of the thesis**). Then, some values were determined: the R_M and the capacity factor $\log k$, using the equation (1) and the equation (2):

$$\log k = \log (t_R - t_0) / t_0 \quad (2)$$

where R_f is the retention factor, t_R is the retention time appropriate to the solution; t_0 is the dead time.

In the analysis by thin-layer chromatography on reversed- phase, from the R_M 's representation in the graph depending on the concentration of methanol in the mobile phase, a linear dependence was observed, dependence expressed by the equation $y = ax + b$, where y is the R_M , b is the originally ordered and a is the slope. Thus, from the equation of the slope corresponding to each studied compound, there have been expressed the originally ordered (R_{M0}) and slope (S). Report of the two (R_{M0} and S) expresses chromatographic hidrofobicity index (ϕ_0). Values of the three sizes are shown in **Table 3.6** (the number of the table corresponds to the notation in the thesis):

Table 3.6 Calculated values of the parameters R_{M0} , S and φ_0

Nr	R_{M0}	S	$\varphi_0 = -R_{M0}/S$
1	1.939	-0.03	65
2	1.07	-0.02	54
3	1.89	-0.03	73
4	1.08	-0.02	54
5	3.00	-0.04	75
6	3.04	-0.04	76
7	2.84	-0.04	71
8	2.63	-0.03	88

Legend: R_{M0} – intercept, S - slope; φ_0 – chromatographic hydrophobicity index

Following the statistical evaluation of regression equations corresponding to the eight p-toluene-sulfonyl-hidrazino-thiazoles, it was found that all results are statistically significant, so that the size of experimental data R_{M0} , S and φ_0 can be considered lipophilicity indicators and will be included in the analysis of structure–activity type compounds .

Similarly, analyzing the eight p-toluensulfonyl-hidrazino-thiazole compounds by liquid column chromatography with reversed -phase, all compounds showed regular behavior, their retention decreased with increasing of the fraction of methanol in the mobile phase. Also, there was a linear dependence between the capacity factor ($\log k$) and the concentration of methanol in the mobile phase, a dependence expressed by specific equations of regression for each compound. From the regression equations were expressed the originally ordered ($\log K_{ow}$), slope S, and the chromatographic hydrophobicity index (φ_0). The values of these quantities are presented in **Table 3.8** (the number of the table corresponds to the notation in the thesis):

Table 3.8 Calculated values of the parameters log K_{ow}, S and φ₀

Nr	Log K _{ow}	S	Log K _{ow} /S
1	3.40	-0.047	72
2	3.76	-0.051	74
3	3.67	-0.051	72
4	2.45	-0.043	57
5	2.57	-0.036	71
6	3.27	-0.049	67
7	4.11	-0.051	81
8	0.78	-0.006	130

Legend: Log K_{ow} – intercept, S - slope, φ₀ - chromatographic hydrofobicity index

Statistical analysis indicates that in the case of the analysis of liquid chromatography with reversed- phases all results are statistically significant so that the log sizes K_{ow}, S and φ₀ can be considered experimental values which will be correlated with theoretical values provided by various algorithms through computational methods in order to determine the biological potential of the eight p-toluensulfonil-hidrazino-thiazole compounds. Modeling the molecular structure of each compound in the series of p-toluensulfonil-hidrazino-thiazoles through a computerized method [40], allows the selection of a number of theoretical descriptors. Theoretical descriptors selected are presented in **Table 3.10** (the table number corresponds to the notation in the thesis):

Table 3.10 Descriptors calculated from the structure using ChemBio3D Ultra 12.0 program

COMPOUND	OBSERVED MEASURES	CALCULATED MEASURES						
	Mw	Ov	PC	BI	CC	MTI	PSA	WI
1	365.47036	1.4683	2.6619	483894	24	9082	70.05	1222
2	401.88824	1.4781	1.6840	593742	25	9608	87.12	1385
3	409.47986	1.4941	1.4439	848027	27	12170	104.19	1700
4	325.4065	1.5172	1.1579	309443	21	7411	87.63	1014
5	345.4392	1.5594	2.9340	384202	23	10251	70.56	1349
6	355.43248	1.5687	1.9550	484499	23	9580	96.86	1330
7	429.51256	1.5311	3.2200	956446	29	15944	87.12	2151
8	350.84634	1.5098	2.4014	321444	22	8511	65.85	1230

Legend: Mw- molecular mass, Ov - ovality, PC - partition coefficient, BI – Balaiban index, CC - the number of groups; MTI - molecular topological index, PSA - polar surface area, WI - Wiener index

In order to analyze the structure - activity relation, we studied the correlation between experimentally determined lipophilicity indicators and descriptors selected through a theoretical specific computational methods [41]. In the study, each descriptor is considered experimental by one dependent variable and the descriptors are theoretically independent variables. We studied the correlation of each experimental descriptor with theoretical descriptors, and different models occurred.

From analysis of regression data, it was found that the same theoretical descriptors correlate with the same experimental descriptors for the series of eight experimental compounds of p-toluensulfonil-hidrazino-thiazole. In other words, R_{M0} correlates with: Wiener index, ovality, polar surface area, molecular topological index and Balaban index. The same theoretical descriptors correlates with slope S, resulted from the regression line equation of RP-TLC analysis. The intercept, $\log K_{0w}$, correlates with the Wiener index, polar surface area and molecular topological index, the same descriptors to which the slope resulted from the regression equation corresponding to the RP-HPLC analysis, correlates. These results confirm that for the compounds from the p-toluensulfonil-hidrazino-thiazole class the expression of the experimental lipophilicity descriptors can be found with similar results, both by thin- layer chromatography on reversed- phase, and by high performance liquid chromatography with reversed-phase [42].

The chromatographic hydrofobicity index (ϕ_0) can be expressed by topological measurements, but the expression is not satisfactory.

4.2 The study of lipofilicity for 12 newly synthesized selenazol derivatives by thin -layer chromatography with reversed- phases

Due to the biological activity of heterocyclic compounds with selenium, activity that was demonstrated as a result of experimental researches, it was found that the presence of selenium in plants can produce poisoning, but at the same time, the presence of selenium in some selenoproteins is considered an essential nutrient. Also, heterocyclic compounds with selenium have anticancer, antibacterial and antiviral activity [43-45]. Compounds analyzed in this study were synthesized by a group of chemists from the Department of Organic Chemistry, University of Medicine and Pharmacy "Iulius Hațieganu" in Cluj - Napoca, and the structures of the synthesized compounds were confirmed using spectra IR, ¹H-NMR, ¹³C-NMR, COSY, HMQC, HMBC, ⁷⁷Se-NMR and MS.

This study considers the prediction of lipophilicity for a series of 12 compounds of selenazol type by using the thin- layer chromatography with reversed- phase method and the correlation of the data obtained with theoretical data.

Experimental data show a linear increase in R_f values with the concentration of the methanol from the mobile phase which shows that the 12 analyzed selenazoles had a regular behavior. R_M values calculated on the basis of the R_f retention factor for the 12 selenazoles were extrapolated to 0% concentration of methanol in the mobile phase resulting the corresponding values R_{M0} . Linear relationship between R_M values and the concentration of methanol in the mobile phase is described by regression equations (**Table 4.3** of the thesis) specific for each analyzed compound together with some statistical estimators ($t_{\text{intercept}}$, T_x , F), on this base being established the significance of coefficients, and the regression equation in this model. In this respect, by analyzing the value of the statistic parameter t (coefficients) and F (equation), it was found that all results are statistically significant because for all 12 compounds considered in this study, the accepted probability of error for the linear model is less than 5%. Although all results are statistically significant, there are two components of the 12 studied that present the risk of error because the error probability approaches the threshold of 5%. This situation

refers to compound 9, which presents the risk of error, accepting the linear model given by the equation $y = -0.0254x + 2.3333$ 3.3%. Even in this case, the worst, we can not reject the hypothesis of linear association. In a similar situation, but less likely to be in error compared to compound 9, is compound 12, which linear model is represented by the equation $y = -0.0319x + 2.3255$. The risk of being in error in this case is 1.03%. Accepting the hypothesis of linear association for the 12 selenazoles, we were led to the predictions of the R_{M0} values (R_M extrapolated to 0% methanol in the mobile phase) and S (slope), values that are given in **Table 4.5** together with 95% probability interval, as shown in the statistical analysis of the obtained regression model.

Table 4.5 R_{M0} , S and ϕ_0 for the 12 studied selenazoles

1	3.49	-0.038	92
2	2.63	-0.030	87
3	2.00	-0.024	84
4	2.17	-0.024	91
5	1.63	-0.019	86
6	2.68	-0.030	91
7	1.57	-0.018	88
8	3.57	-0.040	90
9	2.73	-0.035	77
10	3.00	-0.031	98
11	2.83	-0.029	99
12	2.32	-0.032	73

Legend: R_{M0} – intercept, S - slope, ϕ_0 - chromatographic hydrofobicity index

Based on the analysis of the model presented in the study of the 12 selenazoles, the values obtained for R_{M0} , S and ϕ_0 can be considered experimental values and can be correlated with theoretical values given by different algorithms through computational methods in order to establish the biological potential for the 12 selenazoles.

For the prediction of lipophilicity for the 12 selenazoles, we modeled the structures of the compounds [40], and then, based on this structure, several theoretical descriptors were selected. Out of all the descriptors, there were omitted those which had invalid numeric values for some compounds. Theoretical descriptors which were selected and their values are shown in **Table 4.7** (the table number corresponds to the notation in the thesis):

**Table 4.7 Descriptors calculated from the structure with the program Ultra 12.0
ChemBio3D**

COMPOUND	OBSERVED MEASURES	CALCULATED MEASURES						
	Mw	Ov	PC	BI	CC	MTI	PSA	WI
1	402.73626	1.3780	4.051	456639	24	10757	45.03	1479
2	333.07526	1.4642	3.765	127148	17	4221	36.75	629
3	340.66688	1.3438	2.222	192004	19	5397	45.03	765
4	356.2805	1.5456	4.791	340378	22	9810	45.98	1302
5	336.2478	1.3701	1.428	249512	20	6533	54.26	900
6	398.31718	1.3853	3.256	561855	25	12509	54.26	1683
7	294.21112	1.4762	2.962	127513	17	4652	45.98	631
8	370.69286	1.5080	4.183	338995	21	7723	63.05	1112
9	226.13716	1.3420	1.911	33242	12	1734	50.41	233
10	256.16314	1.3947	1.830	70519	14	2589	59.64	364
11	276.58162	1.3672	0.543	65734	14	2244	67.15	339
12	468.7008	1.3526	3.4919	464812	24	10816	40.32	1504

Legend: Mw - molecular mass, Ov - ovality, PC - partition coefficient, BI – Balaiban index, CC - the number of groups; MTI - molecular topological index, PSA - polar surface area, WI - Wiener index

The analysis of the structure-activity was established by using the SPSS 19 program and it was analyzed the correlation between the calculated properties from the observed properties (**Table 4.5**) and the calculated theoretical descriptors based on chemical structure (**Table 4.6**). In this sense, each experimentally determined indicator was compared with theoretical descriptors. The suggested algorithm is due to choose the best predictors for experimental determined quantities [41].

Following the regression analysis data, it was observed that of the three experimental descriptors included in this study, R_{M0} and S have the best chromatographic determinations compared to the hydrofobicity index, φ_0 . R_{M0} correlates with four theoretical descriptors, S correlates with five and φ_0 with one.

This observation indicates that these correlations are not accidental. Thus, sizes R_{M0} and S can be expressed in topological measurements, while φ_0 can be expressed in these measurements, but the expression is not satisfactory.

It is known that the synthesis of a compound lasts and can provide , with the probability given by the coefficient of determination, the measure by which the newly synthesized compound could meet the objective set in terms of the values R_{M0} , S and φ_0 .

Results obtained on the same compounds, but using other descriptors (ACD / Lab Software, Toronto, Canada) were published in a specialized magazine [46].

4.3 The study of lipophilicity for 17 newly synthesized selenazol derivatives by reversed-phases liquid chromatography

In this chapter we analyzed the lipophilicity prediction for a series of 17 compounds of selenazol type by high performance reversed-phases liquid chromatography and the correlation of the data obtained with theoretical data provided by computational methods .

Following the chromatographic experiment, the retention times (t_R) were measured at a temperature of 25 C degrees and then, based on retention times, it was determined the capacity factor ($\log k$) for each series consisting of the 17 selenazoles. Graphical representation of the capacity factor ($\log k$) depending on methanol content in the mobile phase (% MeOH) showed a linear dependence and allowed determination of the originally ordered- $\log K_{ow}$, the slope index S and the hydrofobicity index , ϕ_0 . The values corresponding to the sizes $\log K_{ow}$, S and ϕ_0 are presented in **Table 5.4** (the table number corresponds to the notation in the thesis):

Table 5.4 LogKow calculated values of the parameters, S and ϕ_0

COMPOUND	Log K _{ow}	S	ϕ_0
1.	5.21	-0.055	94
2.	4.31	-0.052	83
3.	3.99	-0.047	85
4.	4.76	-0.056	85
5.	4.72	-0.062	76
6.	4.87	-0.055	89
7.	3.54	-0.047	75
8.	4.22	-0.047	90
9.	2.20	-0.040	55
10.	2.82	0.044	64
11.	3.81	-0.051	75
12.	2.31	-0.044	53
13.	2.20	-0.036	61
14.	1.68	-0.032	53
15.	3.08	-0.046	67
16.	4.15	-0.055	75
17.	1.20	-0.028	43

Legend: log Kow – intercept, S-slope, ϕ_0 - chromatographic hydrofobicity index

After statistical evaluation of the regression equations corresponding to the 17 selenazol derivatives, the experimental sizes $\log K_{0w}$, S and φ_0 were included in the analysis of structure-activity and are considered lipophilicity indicators for RP-HPLC.

The existence of a large number of computational programs allows the expression of a great variety of molecular descriptors. Thus, based on 3D structures obtained through the program ChemBio3D Ultra 12 [39], we achieved a number of 8 theoretical descriptors for each structure and they are presented in **Table 5.6** (the table number corresponds to the notation in the thesis).

Table 5.6 Descriptors calculated from the structure with the program Ultra 12.0 ChemBio3D

COM- POUND	OBSERVED MEASURES	CALCULATED MEASURES						
	Mw	Ov	PC	BI	C C	MTI	PSA	WI
1.	402.73626	1.3780	4.0510	456639	24	10757	45.03	1479
2.	333.07526	1.4642	3.7651	127148	17	4221	36.75	629
3.	340.66688	1.3438	2.2220	192004	19	5397	45.03	765
4.	356.2805	1.5456	4.7918	340378	22	9810	45.98	1302
5.	336.2478	1.3701	1.4280	249512	20	6533	54.26	900
6.	398.31718	1.3853	3.2569	561855	25	12509	54.26	1683
7.	294.21112	1.4762	2.9627	127513	17	4652	45.98	631
8.	370.69286	1.5080	4.1834	338995	21	7723	63.05	1112
9.	280.18454	1.4269	0.8199	89013	16	3685	53.49	495
10.	314.6296	1.4473	1.6035	119479	17	4118	53.49	591
11.	376.69898	1.5384	3.43255	323532	22	8924	53.49	1237
12.	226.13716	1.3420	1.9110	33242	12	1734	50.41	233
13.	260.58222	1.3610	2.6240	48841	13	1994	50.41	292
14.	256.16314	1.3947	1.8300	70519	14	2589	59.64	364
15.	309.2489	1.4342	2.3529	121597	17	4345	62.77	603
16.	468.7008	1.3526	3.4919	464812	24	10816	40.32	1504
17.	276.58162	1.3672	0.54299	65734	14	2244	67.15	339

Legend: Mw- molecular mass, Ov - ovality, PC - partition coefficient, BI – Balaiban index, CC –the number of groups; MTI - molecular topological index, PSA - polar surface area, WI - Wiener index

The proposed method, SPSS has enabled the establishment of a correlation between experimental descriptors determined by chromatographic methods and theoretical descriptors provided by other computational method. Thus, one of the

experimental descriptors represents the dependent variable, and the ones provided by the program based on the spatial structure are the independent variables which together make up the first model whose correlation will be considered.

It begins by estimating the full model and then, in a number of successive steps, insignificant variables are deleted from the model. At each step, based on a partial F test, is deleted that variable which has the critical probability. The process stops when no variable can be eliminated. The criterion involves the establishing of a removal threshold and the consideration of only the critical variables that have the critical probability higher than this threshold. This type of selection is called retrograde selection or the method of the gradually selection of insignificant variables. The models obtained are estimated by regression analysis. In the case of the 17 selenazoles, the analysis of the regression data obtained indicates that the originally ordered ($\log K_{ow}$) and the chromatographic hydrofobicity index (ϕ_0) can be expressed by topological measurements, while the slope (S) can be expressed from the data, but the expression is not satisfactory. Experimental data obtained were correlated to theoretical descriptors provided by another database, the results obtained were accepted for publication in a specialized magazine [47].

4.4 The study of lipophilicity for 14 new phenothiazin-hidrazino-thiazole derivatives by thin-layer chromatography method

Thanks to a very broad therapeutic applications of compounds of phenothiazin-hidrazino-thiazole type [48, 49], it was necessary to complete the properties already known by a lipophilicity study.

The theme of this study is to determine the lipophilicity of the compounds in the class of phenothiazin-hidrazino-thiazole by thin-layer with reversed-phase chromatography and evidence the experimental descriptors that correlate with theoretical descriptors provided by computational methods.

The lipophilicity of the 14 phenothiazin-hidrazino-thiazole derivatives was investigated by thin-layer with reversed-phase chromatography method. For this study, we worked on silica gel plates of the RP-18WF254 type and the mobile phase was a mixture of methanol and water, the proportion of methanol in the mixture ranging from 70% to 90%. From experimental observations, there were evaluated the retention coefficient values, R_f for the 14 phenothiazin-hidrazino-thiazoles studied. It was found that, while the concentration of methanol in the mobile phase increases, the R_f value increases, too, fact that indicates a regular behavior for all 14 phenothiazin -hidrazino-thiazoles. Based on R_f retention factor, it was determined R_M , and from the graphical representation of R_M size, depending on the content of methanol in the mobile phase, there were determined the R_{M0} intercept, the slope index S and the chromatographic hydrofobicity index, ϕ_0 . Accepting the hypothesis of linear association for the 14 phenothiazin -hidrazino-thiazoles allows prediction of the indicators R_{M0} (R_M extrapolated to 0% MeOH), S (slope) and ϕ_0 (chromatographic hydrofobicity index). Values are presented in **Table 6.5**:

Table 6.5 R_{M0} , S and ϕ_0 for the 14 phenothiazinil- hidrazino-thiazole compounds

Nr	R_{M0}	S	ϕ_0
1	2.57	-0.03	86
2	2.63	-0.023	114
3	3.39	-0.034	100
4	2.95	-0.03	98
5	4.14	-0.043	96
6	4.85	-0.048	101
7	3.40	-0.034	100
8.	2.54	-0.026	98
9	3.00	-0.031	97
10	3.55	-0.036	99
11	1.89	-0.02	95
12	3.67	-0.038	97
13	3.51	-0.035	100
14	3.02	-0.03	101

Legend: R_{M0} – intercept, S - slope, ϕ_0 - chromatographic hydrofobicity index

Analysis of the data presented indicates that the values obtained for R_{M0} , S and ϕ_0 can be considered experimental values that can be included in the analysis of structure-activity to be correlated with the values of theoretical descriptors provided by computational methods in order to determine the biological potential.

Three-dimensional structures of the 14 compounds studied from the phenothiazin-hidrazino-thiazole class were represented using the Ultra ChemBio3DDraw 12.0 program [40]. For the prediction of the lipophilicity of the studied compounds, there were selected a number of descriptors (topological, molecular, etc.) by using the ChemBio3Dultra12 program. Out of the total number of descriptors, there were eliminated those who had invalid numeric values. The confirmation of the values of the indicators of lipophilicity experimentally obtained will be done by correlating experimental values to the theoretical values obtained by means of computational methods. The selected theoretical descriptors for structure-activity analysis are presented in **Table 6.7**.

Table 6.7: Descriptors calculated from the structure with 12.0 ChemBio3DUltra program

COM- POUND	OBSERVED MEASURES	CALCULATED MEASURES							
	Mw	LogP	Ov	PC	BI	CC	MTI	PSA	WI
1.	328.45504	3.582	1.5067	4.3839	286709	22	8064	53.65	1100
2.	366.50302	5.725	1.5696	6.1216	455639	25	12326	39.99	1645
3.	408.5397	5.673	1.5857	4.4329	740667	28	15927	48.27	2152
4.	399.9600	6.35	1.5829	6.4465	557354	26	13513	27.63	1868
5.	450.57638	5.041	1.6258	4.2559	1198122	31	20966	65.34	2866
6.	438.56568	5.939	1.6406	6.7725	1116174	30	20733	66.29	2845
7.	480.60236	5.887	1.6708	5.0530	1628325	33	25028	74.57	3458
8.	466.57578	5.55	1.6056	4.1540	1429245	32	23195	74.57	3221
9.	438.56568	5.547	1.5865	5.8296	1157993	30	21480	66.29	2955
10.	424.5391	5.602	1.5674	5.8735	964594	29	19024	66.29	2625
11.	442.98476	6.044	1.5843	4.496	881015	29	17077	48.27	2395
12.	428.5724	7.119	1.6345	7.7206	969627	30	21926	39.99	2878
13.	470.609	7.067	1.6488	6.0320	1410504	33	26345	48.27	3497
14.	408.5397	5.093	1.6006	5.9754	791913	28	16989	57.06	2302

Legend: Mw- molecular mass, Ov - ovality, PC - partition coefficient, BI-Balaiban index, CC -the number of groups; MTI - molecular topological index, PSA - polar surface area, WI - Wiener index

Structure-activity analysis was performed by gradually eliminating the insignificant variables. In this context we studied one by one, the correlation of experimental descriptors to the theoretical descriptors provided by computational methods applying calculation algorithms. From the analysis of regression, it was found that for all three descriptors for experimental determination is rather weak. In other words, in the case of compounds from this series, phenothiazin-hidrazino-thiazoles, all three experimental quantities can be expressed in topological measurements, but expressions are not satisfactory. It may be noted that, out of the three experimental descriptors reviewed, the best correlation was between R_{M0} and ϕ_0 . In the same way, the number of descriptors that best correlation is achieved with, differs. R_{M0} correlates with one theoretical descriptor (Ov), while ϕ_0 correlates five theoretical descriptors (WI, DC, MW, BI, PC).

5 CONCLUSIONS AND RECOMMENDATIONS

Conclusion

÷ In the same series of compounds such as the p-toluensulfonil-hidrazino-thiazole type that we analyzed, both by RP-TLC and by RP-HPLC, the same theoretical descriptors correlated with experimental descriptors specific to the analysis (R_{M0} and S , respectively, and $\log K_{ow}$, S).

Recommendation

÷ To estimate the lipophilicity of the p-toluensulfonil-hidrazino-thiazoles can be used as working techniques both thin-layer chromatography and high performance liquid chromatography.

Conclusion

÷ The values obtained for R_{M0} , S and ϕ_0 for the 12 selenazoles and evaluated by a regression analysis allow us to say that they can be considered experimental values that will be correlated with theoretical values provided by means of computational methods algorithms, such as ChemBio3DUltra12.0

Recommendation

÷ The regression analysis can be used to study if the sizes considered as descriptors of lipophilicity for selenazoles are or are not statistically significant.

Conclusion

÷ For 17 selenazoles analyzed by high performance liquid chromatography was found a linear dependence of the capacity factor, $\log k$, the concentration of methanol in mobile phase composition, so that the regression equations expressed for each compound that was considered have the correlation coefficient greater than 0.9 in all cases.

Recommendation

÷ To determine the quality of experimental predictor for lipophilicity for a size ($\log K_{ow}$), it is prior recommended the study of the dependence of the capacity factor of the concentration of the organic solvent in the mobile phase.

Conclusion

÷The structure-activity analysis of the 17 selenazoles that were analyzed by high performance liquid chromatography revealed that between $\log K_{ow}$, respectively ϕ_0 –the chromatographic hydrofobicity index and theoretical descriptors established a very good correlation, these two descriptors can be considered for this model predictors of lipophilicity, while S slope can not be considered a satisfactory predictor of lipophilicity for the compounds in this series, as between S slope and the provide theoretical descriptors based on the structure of various computational methods established a correlation which is unsatisfactory.

Recommendation

÷It is recommended to estimate the lipophilicity for the 17 selenazoles analyzed by high performance liquid chromatography using the two descriptors: $\log K_{ow}$ and ϕ_0 .

Conclusion

÷In the case of the the series consisting of the 14 derivatives of phenothiazin-hidrazino-thiazole,the structure-activity analysis performed by the gradual elimination of insignificant variables and evaluated by regression analysis indicates that all three descriptors considered in this study as experimental ,measurements are quite poor. In other words, all three experimental quantities can be expressed in topological measurements, but the expressions are not satisfactory.

Recommendation

÷For the phenothiazin- hidrazino- thiazole series of compounds, in order to be correlated with the experimental descriptors set, is recommended the use of other theoretical descriptors provided by computational methods other than those in this study.

Conclusion

÷The results obtained show that, both the analysis by thin -layer chromatography and high performance reversed- phase liquid chromatography are techniques to estimate lipophilicity , even if there were used chromatographic plates of RP-18WF254 type or column C-18 ,the results being comparable for compounds belonging to the same series.

Recommendation

÷For the p-toluensulfonil-hidrazino-thiazole type compounds, selenazoles and phenothiazin-hidrazino-thiazoles can be used, as working techniques to estimate lipophilicity, the thin-layer chromatography method with reversed-phase and the liquid chromatography with reversed-phase method, and as stationary phases can be used plates of RP-WF254 type, respectively C-18 columns.

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LISTS OF THE PAPERS THAT WERE ACCEPTED FOR PUBLICATION

1 A. Cozma, L. Vlase, A. Ignat, V. Zaharia, S. Gocan, N. Grinberg, **Prediction of the lipophilicity of eight new *p*-toluenesulfonyl-hydrazino-thiazole and hydrazine-bis-thiazole derivatives: a comparison between RP-HPTLC and RP-HPLC** *J. Liq. Chromatogr. & RT*, (ID LJLC-2011-0631; accepted for publication)

2 A. Cozma, L. Vlase, A. Ignat, S. Gocan, C. Marutoiu, A. Fodor, **Prediction of the Lipophilicity of 17 newly selenazoly derivatives by reversed-phase high performance liquid chromatography** *Rev. Chim. (București)*, **63** (2012) (accepted for publication)

3 A. Cozma, L. Vlase, A. Ignat, V. Zaharia, S. Gocan, N. Grinberg, **Prediction of the Lipophilicity of 12 New Synthesized Selenazoly Derivatives by Reversed-Phase High Performance Thin-Layer Chromatography”** *J. Chromat. Sci.* (MS ID JCS-11-005.R1; accepted for publication).

4 **A comparative study of the lipophilicity for eight new compounds by high performance liquid chromatography and thin layer chromatography** – public presentation at the Science Faculty Conference, Universitatea din Oradea, 11 noiembrie 2011