

THE EVALUATION OF THE LIPOPHILIC PROPERTIES OF SOME THIAZOLYL-OXADIAZOLINES WITH ANTIINFLAMMATORY ACTIVITY

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Abstract

The lipophilicity of 15 new thiazolyl-oxadiazolines was determined, using the principal component analysis (PCA) method. Retention indices were determined by reversed phased thin-layer chromatography (RPTLC). By comparing the results obtained from the lipophilicity data with the anti-inflammatory activity, it was revealed that, for a good anti-inflammatory effect, the compounds need to have medium lipophilicity values. Low or high lipophilicity values are associated with a decrease or abolition of the anti-inflammatory effect.

Rezumat

A fost evaluată lipofilia a 15 noi tiazolil-oxadiazoline prin utilizarea analizei componentelor principale (PCA). Datele necesare pentru aplicarea acestui model matematic (factorul de retenție R_f) au fost obținute prin utilizarea cromatografiei în strat subțire cu faze inverse. Comparând rezultatele obținute pentru parametrii de lipofilie cu activitatea antiinflamatoare a tiazolil-oxadiazolinelor s-a constatat că pentru un bun efect antiinflamator compușii trebuie să prezinte valori medii ale lipofiliei. Obținerea unor compuși cu valori mari sau foarte mici ale lipofiliei conduce la reducerea până la dispariție a efectului antiinflamator.

Keywords: lipophilicity, principal component analysis, thiazolyl-oxadiazolines

Introduction

The biological activity of an active substance is correlated with its physicochemical properties, such as octanol/water partition coefficient, electronic density, polarity etc. In order to be pharmacologically or biochemically effective, a substance must first reach the action sites in adequate concentrations. For this to take place the substance has to travel through a series of biological membranes in which lipids play a crucial role as they are the major constituents. The lipophilic character of an active molecule, known as lipophilicity, was defined as the ability of a compound

to penetrate through hydrophobic barriers in order to get from the site of administration to the action site [20].

The lipophilic character of a compound can be expressed by the partition coefficient P that represents the ratio of concentrations of the compound in the two phases of a mixture of 2 immiscible solvents. This parameter can be determined experimentally, employing an extraction technique, or it can be calculated by using a series of mathematical models [1-3,7,10].

Because the extraction technique requires a large amount of solvents and an extended analysis time and because the mathematical models usually employed are incomplete, other techniques were used in order to obtain superior parameters that estimate a compound's lipophilicity, namely the use of logarithmic R_f (retention factor), usually termed R_M . By applying the Batte-Smith and Westall equation to the R_f values, the logarithmic R_M values may be obtained (table 1) [4,5,10,14,24]:

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

The use of R_M values obtained from thin layer chromatography as hydrophobic parameters for correlation with biological activity appears to be more suitable as a support of the lipophilicity scale [22,23]. The advantages of chromatographic methods are the small quantity of compounds needed for the determination and the rapidity and simplicity of the method. The R_M value determined by use of reversed-phase thin-layer chromatography (RPTLC), generally depends linearly on the concentration of the organic component of the mobile phase. By extrapolation of the R_M value to zero organic component concentration, a new parameter (R_{M0}) was obtained, which can be considered as an estimation of the partitioning of compounds between nonpolar stationary phase and the aqueous system and hence a way to estimate the lipophilicity of the compounds. Additionally, it has been stated that not only the R_{M0} , but also the slope, b , of the regression line (2) can be used as an estimation of lipophilicity. The slope is regarded as a characteristic of the specific hydrophobic surface area of the compounds [6,8].

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

The aim of the present study is to evaluate the lipophilic properties of some thiazolyl-oxadiazolines with anti-inflammatory potential, by using the principal component analysis method (PCA). PCA, also known as eigenvector analysis, eigenvector decomposition or Karhunen-Loeve expansion, involves a mathematical procedure that transforms a number of

possibly correlated variables into a smaller number of uncorrelated variables called principal components, PC (factor scores or scores). The first principal component PC_1 accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. Two or three principal components usually provide a good summary of all the original variables. Scores plots are very useful as a display tool for examining the relationships between objects, and looking for trends, groupings or outliers [9,13].

This data processing was possible due to the fact that the R_f chromatographic parameter can be considered as a combination of physico-chemical and molecular properties (solubility, acido-basic character, polarity, steric and electronic interactions), that have a decisive influence on the lipophilic character of a compound. Moreover, PCA allows a generalized view over the behavior of a series of compounds in relation with a given system, provided that all compounds are being simultaneously investigated by chromatography. This procedure leads to a new series of parameters capable to quantify the behaviour of the investigated compounds, in certain conditions, and capable to quantitatively assess the lipophilic character of the compounds.

Material and methods

The data required for this mathematical model was obtained by RPTLC. A number of 15 compounds were selected and subjected to RPTLC in order to obtain the parameters necessary for the estimation of the lipophilic properties [22,23]. The synthesis of these compounds was previously reported and the structures are presented in Figure 1 [15, 16, 18, 19].

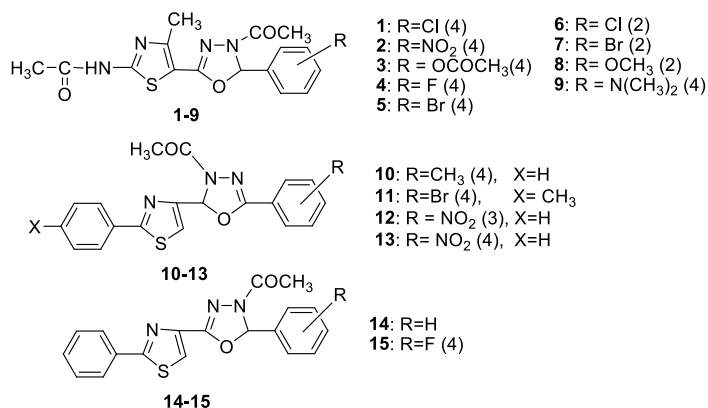


Figure 1

The structures of the thiazolyl-oxadiazolines derivatives 1-15

The chromatographic behaviour of the selected compounds was studied by using standard RP-18W/UV254 (20 x 20 cm) chromatographic plates, with a chemically bound C₁₈ stationary phase, acquired from Machery-Nagel (Duren, Germania) [22,23].

We used 0.1% methanolic solutions of the investigated compounds. The solutions were manually applied as spots at 15 mm from the base and 10 mm from the edges of the plate; the distance between consecutive spots was 6 mm. The samples were applied using standardized, 1 µL chromatographic micropipettes. The mobile phase consisted of a mixture of water and methanol in various proportions ranging from 50% to 75% (v/v). The difference between the concentrations of two consecutive elution mixtures was of 5-10%. This domain of concentration was determined through previous studies, which led to the conclusion that all the tested compounds have significant R_f values within this range of concentration. The distance travelled by the mobile phase front was 7 cm for the 50% methanol solution and 8 cm for all the rest.

We used a standard developing chamber, over-saturated with solvent vapor. The saturation period was 15 minutes. The detection of spots and determination of the traveling distance was performed by exposure to UV light at 254 nm; in these conditions all compounds have a light-blue fluorescence. The traveling distance was measured manually and was used to calculate the R_f values. The R_f values were determined for every compound in each of the conditions of the 4 mixtures used as mobile phases.

PCA has been performed on the retention data matrix by the use a computer program [22,23,12, 21].

In order to correctly assess the initial data set, the first three principal components were retained; these explained 96.31% of the total variance (information) from the initial data. The first component explains 60.38% of the total variance, the second 29.33% and the third only 6.3%.

Results and discussion

By applying the Batte-Smith and Westall equation to the R_f values, the logarithmic R_M values may be obtained (table 1)

Due to the linear dependency between the R_M value and the organic component's concentration in the mobile phase [c], the values for R_{M0} and b were determined by extrapolation to zero; the results of regression analysis are shown in table II. These values give a quantitative estimation for the distribution of the investigated compounds between a non-polar phase (a chemically bounded reversed stationary phase) that represents the biological

membranes, and a polar phase that represents the extracellular aqueous environment.

Table I

The retention parameters for compounds 1-15

Comp. no.	Methanol% : water% (v:v)							
	50%:50%		55%:45%		65%:35%		75%:25%	
	R _f	R _M	R _f	R _M	R _f	R _M	R _f	R _M
1	0.4437	0.09822	0.502	-0.00347	0.5308	-0.05357	0.6845	-0.33637
2	0.5	0.08715	0.5555	-0.09681	0.6604	-0.28884	0.7678	-0.51939
3	0.4812	0.03264	0.5185	-0.03215	0.6049	-0.18498	0.7023	-0.37274
4	0.4875	0.02179	0.501	-0.00174	0.574	-0.1295	0.7142	-0.39776
5	0.3625	0.24512	0.4444	0.09698	0.4629	0.064	0.6547	-0.27785
6	0.4	0.17601	0.4876	0.02154	0.5802	-0.14054	0.6726	-0.31268
7	0.425	0.13129	0.4815	0.03215	0.5185	-0.03215	0.6785	-0.32437
8	0.475	0.04346	0.5247	-0.04294	0.5617	-0.10773	0.6964	-0.36056
9	0.4375	0.10914	0.5123	-0.02137	0.5493	-0.08592	0.6392	-0.24837
10	0.4462	0.09383	0.4815	0.03215	0.5247	-0.04294	0.5416	-0.07243
11	0.375	0.22189	0.574	-0.1295	0.7037	-0.37566	0.8392	-0.71758
12	0.4562	0.07624	0.4753	0.04294	0.505	-0.00869	0.5178	-0.03093
13	0.4475	0.09159	0.4629	0.06456	0.4975	0.00434	0.5123	-0.02137
14	0.3062	0.35529	0.4568	0.07523	0.6666	-0.3009	0.7976	-0.59557
15	0.2625	0.44863	0.4691	0.05374	0.5062	-0.01077	0.5833	-0.14607

Table II

Regression data and scores on the first three principal components for the investigated compounds

Comp. no.	R_{M0}	b	r^2	PC ₁	PC ₂	PC ₃
1	0.908	-1.602	0.911	-0.067	0.442	0.156
2	1.376	-2.396	0.832	2.371	0.530	-0.259
3	0.855	-1.623	0.995	0.967	0.902	0.242
4	0.901	-1.678	0.935	0.509	0.859	0.672
5	1.185	-1.882	0.891	-1.783	-0.989	0.501
6	1.089	-1.882	0.985	0.033	0.371	0.101
7	0.991	-1.697	0.922	-0.538	0.034	0.382
8	0.810	-1.514	0.932	0.645	0.986	0.019
9	0.741	-1.310	0.956	-0.025	0.533	-0.338
10	0.403	-0.654	0.935	-1.278	0.672	-0.277
11	1.903	-3.515	0.966	3.385	-0.607	-0.671
12	0.283	-0.430	0.957	-1.675	0.857	-0.243
13	0.710	-1.181	0.984	-1.969	0.651	-0.00
14	2.173	-3.738	0.986	0.972	-2.468	0.53
15	0.465	-0.784	0.888	-1.546	-2.032	-1.039

From the analysis of the obtained data we can observe that there is a linear dependency between the values of R_{M0} (lipophilic parameter) and the values of b (specific hydrophobic surface). This linear dependency clearly shows that the thiazolyl-oxadiazolines form a homologous series of compounds. Also, the values obtained for the r^2 parameter show that there is a high degree of linear dependency between the R_M parameter and the concentrations of the organic component from the mobile phase. This linearity is a compelling argument to the fact that all chromatographic experiments were conducted appropriately and moreover, it allows us to

assert that R_{M0} values can be considered a parameter that is capable to estimate the lipophilic character of the studied compounds.

Scores on the first three principal components are also presented in table II. A high correlation was observed between R_{M0} values and the scores on the first principal component of the compounds (figure 2), the compounds: **5** (4N-acetyl- 2[2'-(acetyl-amino)-4'-methyl-5'-thiazolyl]-5-pBr-phenyl- Δ_2 -oxadiazoline), **13** (4-N-acetyl-2-p-nitro-phenyl-5[-(2'-phenyl-thiazol-4'-yl)]- Δ_2 -1,3,4-oxadiazoline) and **14** (4-N-acetyl-5-phenyl-2[-(2'-phenyl-thiazol-4'-yl)]- Δ_2 -1,3,4-oxadiazoline), constituted an exception from this trend.

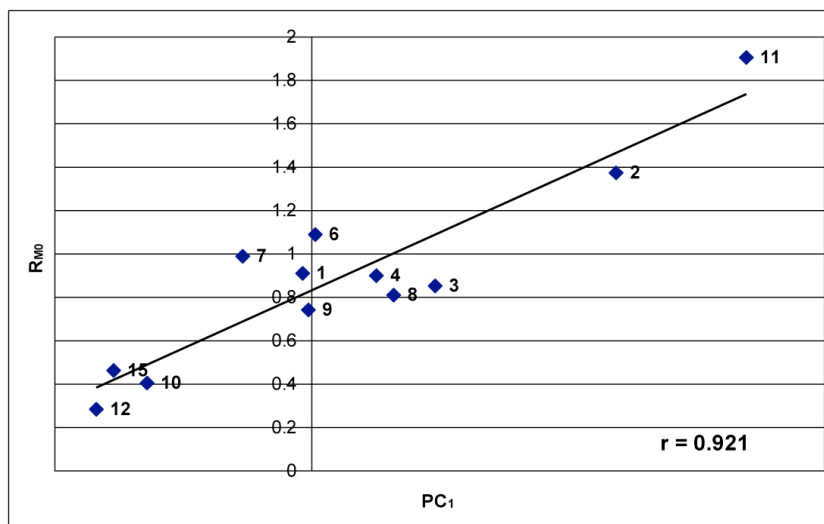


Figure 2
Relationship between R_{M0} and PC₁

On the basis of these findings and as it can be seen from data provided in table II, the scores on the first principal component can replace efficiently the R_{M0} values in the estimation experiments of the lipophilicity. The values obtained for the principal components served to create a relative lipophilic scale in which a compound with a high lipophilic character is characterized by a high value of the PC₁ parameter. Also, graphing scores onto the space described by PC₁, PC₂ and PC₃ we obtain “the congeneric lipophilicity space” (Figure 3) [22,23]. It appears clearly that the studied compounds form two congeneric classes.

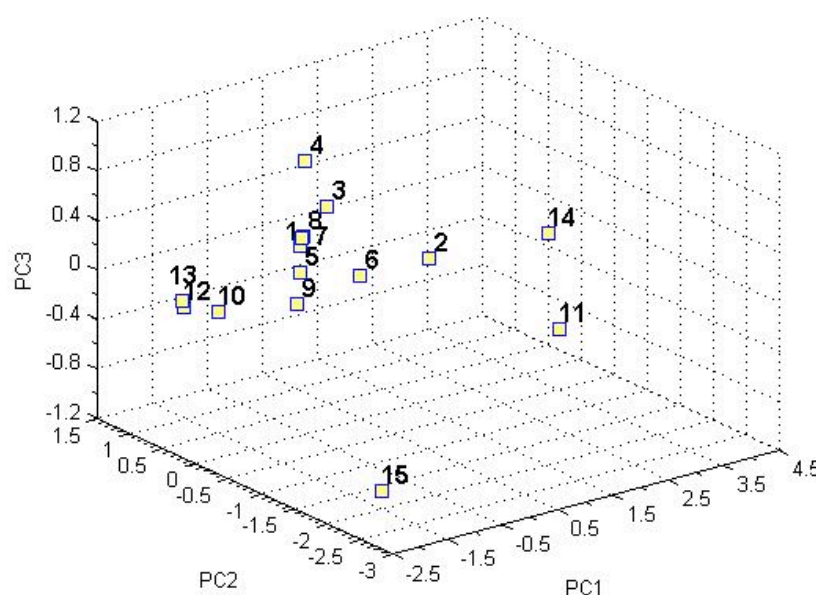


Figure 3

3-D scatterplot of scores corresponding to the first three principal components

The two congeneric classes that became distinctly obvious are:

- class 1: compounds: 4N-acetyl- 2[2'-(acetyl-amino)-4'-methyl-5'-thiazolyl]-5-aryl- Δ_2 -oxadiazolines **1-9** and 4-N-acetyl-2-R-phenyl-5[-(2'-phenyl-thiazol-4'-yl)]- Δ_2 -1,3,4-oxadiazolines **10,12,13**
- class 2: compounds: **14** (4-N-acetyl-5-phenyl-2[-(2'-phenyl-thiazol-4'-yl)]- Δ_2 -1,3,4-oxadiazoline) and **11** (4-N-acetyl-2-p-Br-phenyl-5[-(2'-thiyl-thiazol-4'-yl)]- Δ_2 -1,3,4-oxadiazoline).

We can also observe the presence of compound (**15** - 4-N-acetyl-5-phenyl-2[-(2'-p-F-phenyl-thiazol-4'-yl)]- Δ_2 -1,3,4-oxadiazoline) that does not fit in this mathematical model („outlier”).

These results reveal that the most lipophilic compound is 11, which belongs to the 4-N-acetyl-5-(2'-p-thiyl-thiazol-4'-yl)- Δ_2 -1,3,4-oxadiazoline series and has a bromine substituent in the para position of the phenyl ring situated in the position 2 of the oxadiazoline ring.

Compounds 1-9, belonging to the 4N-acetyl- 2[2'-(acetyl-amino)-4'-methyl-5'-thiazolyl]-5-aryl- Δ_2 -oxadiazoline series have similar values of the lipophilicity parameter; this can be explained through the essential contribution of the molecular backbone to the lipophilic character.

Further on, we decided to establish, if possible, a correlation between the values we obtained for the lipophilic parameters and the anti-inflammatory activity for the compounds that have been tested for it [17]. We considered the values obtained for the phagocytic index, PI (the phagocytic capacity of free leukocytes has been evaluated by the percentage of leukocytes that ingested at least one germ), a parameter that estimates the effect in acute inflammation and those obtained for the concentrations of nitrite/nitrate (as metabolites of NO via the L-arginine-NO metabolic pathway), parameter that estimates the anti-inflammatory effect in both acute and chronic inflammation (table III).

Table III

Correlation between the lipophilic parameters and anti-inflammatory activity parameters

Compound no.	PI % [17]	nitrite/nitrate mg/dL [17]	R_{M0}
1	20	0.00416	0.908
3	25	0.00154	0.855
7	39,6	0.0015	0.991
6	45	0.00184	1.089
2	45	0.0028	1.376
12	50	0.00196	0.283

This correlation of data shows that in order to possess a good anti-inflammatory effect the compounds must have medium lipophilic character (compounds 1, 3, 7). Compounds that have either a high or a very low lipophilic character also have a reduced or absent anti-inflammatory effect (compounds 2, 6, 12). It is also noticeable that a decrease in the lipophilic character of a compound does not influence in any way the compound's ability to reduce the concentrations of nitrite/nitrate.

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References

1. Biagi G.L., Barbaro A.M., Sapone A., Recanatini M.J. – Determination of lipophilicity by means of reversed-phase thin-layer chromatography, *Journal of Chromatography A*, 1994, 669, 246-251;
2. Bieganowska M.L., Petruczynik A., Doraczynska-Szopa A - Comparison of the Retention Behaviour of Some Basic Drugs used Mainly Against Parkinson's Disease in Normal- and Reversed-phase Thin-layer Chromatography., *Chem. Anal. Warsaw*, 1993, 38, 719-824;
3. Bieganowska M.L., Rompala A.– Retention behavior of some phenoxyacetic acid derivatives on silica gel and diol bonded silica gel HPTLC precoated platets, *J. Liquid Chromatogr.*, 1999, 22, (10), 1443-1456;
4. Brown, S.D, Sum, S.T., Despagne, F., Lavine, B.K. - Chemometrics: Fundamental Review, *Anal. Chem.*, 1996, 68, 21-61
5. Brown, S.D., Blank, T.B., Sum, S.T., Weyer, L.G., - Chemometrics: Fundamental Review, *Anal. Chem.*, 1994, 66, 315-359
6. Cserhádi T., Bordás B., Ekiert L., Bojarski J., Effect of layer and eluent characteristics on the reversed-phase thin-layer chromatographic behaviour of some barbituric acid derivatives, *Journal of Chromatography A*, 1984, 287, 385-390;
7. Gocan, S., Optimization of a Quaternary Solvent System for the Separation of 1-(2-Pyrimidyl)-3-Methylpyrazolone Cleavage Products by RP-TLC, *J. Planar Chromatogr.*, 1990, 4, 169-174;
8. Horvath C, Melander W, Molnar I. Solvophobic interactions in liquid chromatography with nonpolar stationary phases. *J Chromatogr* 1976; 125, 129–156;
9. Joliffe I.T., Principal component analysis, Springer Series in Statistics, 2nd ed., Springer, NY, 2002;
10. Kiridena, W., Poole, C.F. - Structure-Driven Retention Model for Solvent Selection and Optimization in Reversed-Phase Thin-Layer Chromatography., *J. Chromatogr. A*, 1998, 802, 335-339;
11. Kiridena, W., Poole, C.F. - Structure-Driven Retention Model for Solvent Selection and Optimization in Reversed-Phase Thin-Layer Chromatography., *J. Chromatogr. A*, 1998, 802, 335-347
12. Mackiewicz A., Ratajczak W., Principal components analysis, *Computer & Geoscience*, 1993, 19, 303-342;
13. Mannhold, R., Cruciani, G., Dross, K., Rekker, R., - Multivariate analysis of experimental and computational descriptors of molecular lipophilicity, *J. of Computed-Aided Molecular Design*, 1998, 12, 573-581;
14. Olsson, M.I., Experimental Designs at the Crossroads of Drug Discovery, Doctoral Dissertation, Umea University, Sweden, 2004
15. Oniga S., Oniga O., Tipericiu B., Palage M., Mureşan A., Ghiran D. Synthesis and antimicrobial activity of some new 5-dithiazolil-2-R-1,2,3- Δ_4 -oxadiazolines derivatives, *Farmacia*, 2000, vol XLVIII, (3), 65-69
16. Oniga S., Oniga O., Chirtoc I., Ionescu M., Tipericiu B., Ghiran D. Obținerea și activitatea antimicrobiană a unor 3-N-acetil-5[2'-(acetil-amino)-4'metil-5'tiazolil]-2-aril- Δ_4 -1,3,4-oxadiazoline, *Farmacia*, 2005, vol. LIII, (1), 28-35;
17. Oniga S., Pârvu A.,E., Tipericiu B., Palage M., Oniga O. - The study of the anti-inflammatory activity of some thiazolyl- Δ_2 1,3,4 oxadiazolines and 5-carboxiethyl-2-hydrazone-4-methyl-thiazole-derivatives, *Farmacia*, 2011, vol.59(1), 44-50
18. Oniga, O., Tipericiu B., Oniga S., Ioanoviciu A., Săndulescu I., Mogoşan C. The Synthesis and anticonvulsant activity of new 2-[1,3,4- Δ_4 -oxadiazoline]-4'-thiazole derivatives, *Farmacia*, 2000, vol XLVIII, (4),77-83;
19. Oniga, O., Oniga S., Tipericiu B., Preda L., Ghiran D., Chirtoc I. The synthesis of some 3N-acetyl-2-R-5-[2'p-X-phenyl-thiazol-4'-yl] Δ_4 -1,3,4-oxadiazolines, *Clujul Medical*, 2000, vol. LXXIII, (2), 293-297;

20. Rekker R.F., Mannhold R., Calculation of Drug Lipophilicity, Weinheim, 1992;
21. Sarbu C., Pop H., A new fuzzy regression algorithm, *Anal.Chem*, 1996, 68, 771-778;
22. Sârbu C., Todor S. – Determination of lipophilicity of some non-steroidal anti-inflammatory agents and their relationships by using principal component analysis based on thin-layer chromatographic retention data, *Chromatogr.*, 1998, 822, 263-269;
23. Sârbu, C., Malawska, B., Evaluation of lipophilicity of piperazine derivatives by thin layer chromatography and principal component analysis, *Journal of Liquid Chromatography & Related Technologies*, 2000, 23, (14), 2143-2154;
24. Steyaert, G., Lisa, G., Gaillard, P., Boss, G., Rezmond, H., Carrupt, P., Testa, B.- Intermolecular forces expressed in 1,2-dichloroethane-water partition coefficients, *J. Chem.Soc., Faradaz Trans.*, 1997, 93, (3), 401-406

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