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Evaluation of lipophilicity of some benzimidazole and benztriazole derivatives by RP HPTLC and PCA

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Abstract

The lipophilic character of some benzimidazole and benztriazole derivatives was studied. The classical R_{Mo} values were compared with the factors scores obtained by principal component analysis (PCA) based also onto the TLC retention data. The very high correlation between the R_{Mo} values and slopes (specific hydrophobic surface area) indicated as usually that this group of compounds could be considered as a homologous series independently of their structural heterogeneity. It is emphasized once again that this procedure can not be a rational and objective way for congeneric studies because always there is a high correlation between slope and intercept. The reliability of the factor scores values as lipophilic indices are shown by their high correlation with the classical R_{Mo} values. In addition, the 'lipophilicity chart' described by the first two components has the effect of separating compounds from each other most effectively from the congeneric aspect point of view. The most lipophilic compounds appeared to be the benzimidazole derivatives.

Keywords: Benzimidazole and benztriazole derivatives; Lipophilicity; QSAR; PCA

1. Introduction

Benzimidazoles and benztriazoles are well known physiologically active compounds. Benzimidazoles, for example, are used as vasodilator drugs and others have antihypertensive, anti-inflammatory, arteriosclerosis inhibiting antifungal and pesticide activity [1–5]. Due to their physiological activity and commercial application, many different benzimidazoles, particularly substituted 2-aminobenzimidazole derivatives, have received much attention during the last decade [6,7]. However, there is very little information found with respect to the toxicokinetics of benztriazoles. Preliminary investigations have shown that newly synthesised 1,2,4-triazole derivatives are biologically active against *Escherichia coli*, *Bacillus subtilis*, *Salmonella enteritidis* and *Aspergillus niger* [8].

Considering the increasing of practical importance—pharmaceutical and toxicological—of aromatic and heterocyclic amines we selected for the study two groups of compounds one of benzimidazole and another of benztriazole derivatives.

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The purpose of this paper is to investigate the feasibility of the scores, obtained by principal component analysis (PCA) using RPTLC retention data, as a measure of lipophilicity in correlation with partition coefficient ($\log P$) and other descriptors of the compounds considered. In addition, the scatterplots of the scores onto plane described by the first two components appear to be very useful having the effect of separating compounds one from each other most effectively, obtaining in this way the 'congeneric lipophilicity chart' of the series.

Quantitative structure–activity relations (QSAR) describe how the molecular structure, in terms of descriptors—lipophilic, electronic and steric—affects the biological activity of a compound [9–12]. Similarly, quantitative structure–retention relations (QSRR) relate these descriptors to chromatographic retention. Finally, the quantitative retention–activity relations (QRAR) imply that conclusions concerning biological activity can be based on chromatographic experiments [13–17]. In this sense, it is considering that the same basic intermolecular actions determine the behavior of chemical compounds in both biological and chromatographic environments. As a consequence, the chromatographic approach has been quite successful in duplicating $\log P$ data derived by traditional 'shake-flask' technique or other procedures [18–23]. The relations themselves are usually based on correlation analysis. For instance, the use of R_M values obtained from various types reversed-phase thin layer chromatography is based on the assumed linear relationship between the molecular parameter (Eq. (1)) and $\log P$.

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

The advantages of TLC methods are the very small amounts of sample needed for the estimation and the less strict requirement of purity because the impurities separate during the chromatographic process. They are rapid and relatively simply, low cost and easy to perform. In addition, we have to stress the dynamic aspect of the chromatographic process and the wide choice of stationary phases and developing solvents.

The R_M value (related to the molecular lipophilicity), determined by using of RPTLC, generally, depends linearly on the concentration of the organic component of the mobile phase:

$$R_M = R_{Mo} + b\varphi \quad (2)$$

where R_M-values were calculated using Eq. (1) and φ is the volume fraction of organic modifier.

Another form of computational analysis used for the correlation of biological activity, structure, and chromatographic retention is PCA [24–31]. By using the multidimensional space described by the different mobile phases, a quantitative model is derived that transforms the axes of the system. The first principal component (PC1) defines as much of the variation in the data as possible. The second principal component (PC2) describes the maximum amount of residual variation after the first PC has been taken into consideration, and so on. By using only a limited number of PCs, the dimensionality of the data space is reduced, thereby simplifying further analysis. In chromatography two principal components are often sufficient to describe most of the retention data variation. Although the PCs are abstract, one of them often shows high correlation with lipophilicity, molecular size, or steric factors, whereas the other PC seems more strongly correlated with dipole–dipole interactions and electronic factors.

2. Experimental

The chromatographic behavior of the compounds was studied on the C_{18} silica gel bonded plates. HPTLC plates (20 × 20 cm) were obtained as a gift from Macherey-Nagel (Düren, Germany). Methanol for chromatography was supplied from Reactivul (Bucharest, Romania). The investigated benzimidazole and benztriazole derivatives were synthesized by procedure described earlier [32]. The 1 mg ml^{-1} of each compound, in Fig. 1, was dissolved in methanol and 3 μl volume of the prepared solution was spotted randomly on the plates.

Chromatograms were developed by ascending technique at room temperature; the developing distance being 8 cm. The mobile phase was mix-

ture of methanol–water with various content of methanol (45–60% (v/v) in steps of 5%) as the studied compounds differed considerably in their retention.

After being developed, the dried plates were examined under UV lamp ($\lambda = 254$ nm).

3. Principal component analysis

PCA [26–31] has been performed on the retention data matrix by the use of a computer program discussed in [25]. It display compounds in a reduced space by finding a direction (first principal component) that best preserves the scatter of the observations (R_F values) in the multidimensional space described by the solvent systems. As usually PCA gives both coordinates (scores) of the studied compounds and the loading of variables (solvents) on the principal components.

The results obtained from the initial chromatographic data using covariance matrix (without autoscaling) can be presented as usually in three panels, although typically there are only two. The first panel shows the table of data statistics; the second is the table of components and the third panel displays the eigenvectors associated with each of the components. Table 1 lists the eigenvalues of the covariance matrix, ordered from largest to smallest, the third column of this table shows the difference between each eigenvalues and the next smaller eigenvalue and the fourth column shows the proportion. These results suggest a significant two component model explaining 99.30% of the total variance (information), considering only the eigenvalues higher than one. The first component explains 97.88% of the total variance, the second 1.42% and the third only 0.55%; the subsequent eigenvalues are just sampling noise.

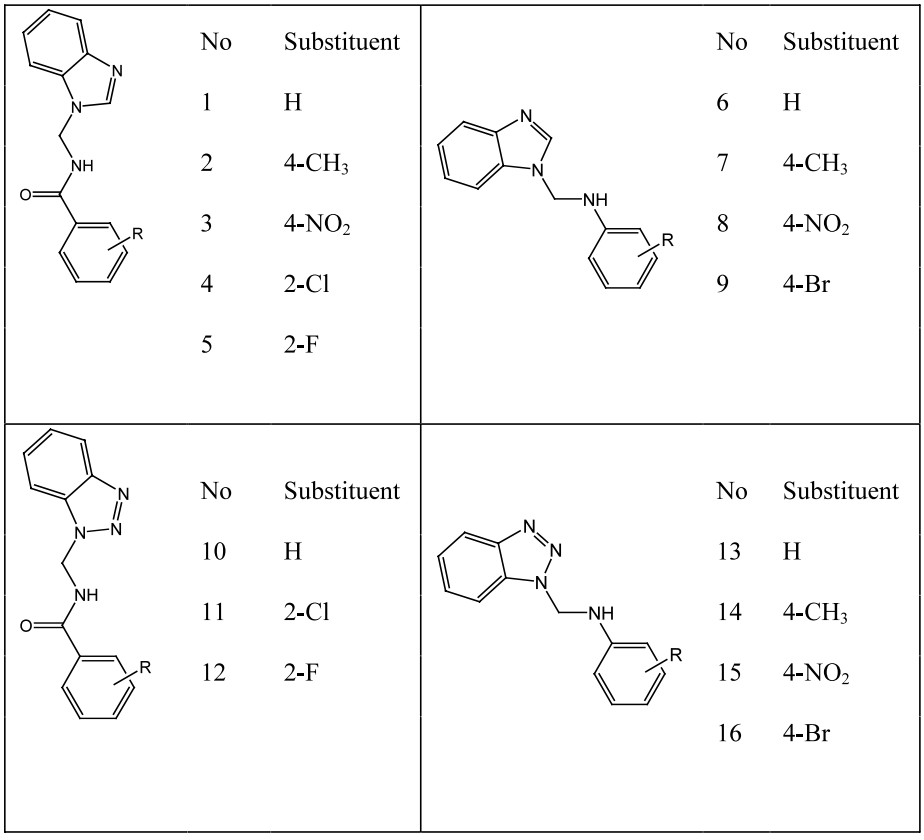

Fig. 1. The chemical structure of the benzimidazole and benztriazole derivatives studied.

Table 1
The eigenvalues and the ratios of the variance explained by the four components using covariance matrix

Component	Eigenvalue	Difference	Proportion (%)	Cumulative (%)
1	0.7637		97.880	97.880
2	0.0111	0.7526	1.417	99.297
3	0.0043	0.0068	0.546	99.843
4	0.0011	0.0032	0.157	100.000

Table 2
Regression data and scores on the first two principal components for the benzimidazole and benztriazole derivatives studied in this paper

Compound	R_{Mo}	b	r	R^2	PC1	PC2
1	2.71	−4.15	−0.9458	0.8945	0.459	−0.218
2	3.15	−4.77	−0.9449	0.8928	0.379	−0.212
3	2.82	−4.21	−0.9592	0.9200	0.401	−0.199
4	2.58	−4.09	−0.9532	0.9086	0.537	−0.241
5	2.74	−4.32	−0.9699	0.9409	0.501	−0.241
6	2.50	−3.98	−0.9638	0.9289	0.556	−0.237
7	2.56	−3.53	−0.9467	0.8962	0.558	−0.219
8	1.23	−2.41	−0.9992	0.9984	1.015	−0.227
9	1.71	−2.75	−0.9677	0.9365	0.691	−0.210
10	2.36	−4.00	−0.9782	0.9568	0.695	−0.228
11	2.15	−3.73	−0.9789	0.9583	0.763	−0.276
12	2.36	−4.00	−0.9782	0.9568	0.695	−0.278
13	0.95	−2.19	−0.8967	0.8041	1.201	−0.212
14	1.93	−3.14	−0.9758	0.9522	0.676	−0.225
15	1.15	−2.15	−0.9253	0.8561	0.952	−0.202
16	1.63	−2.58	−0.9440	0.8912	0.684	−0.198

It is interesting also to mention that when the significance of the component model retained was tested applying the Bartlett's statistics [25], testing the hypothesis that $(p–k)$ eigenvalues in variance–covariance matrix are equal, a model with two components was also selected.

4. Results and discussion

The results of regression analysis using Eq. (2) are compiled in Table 2. The statistics obtained (see also Table 2) illustrate that the linear equation fits in a very good way the experimental data, the linear model explaining over 90% of the total variance (see R^2 values) in the majority of cases. As usually a good correlation has been found also between the R_{Mo} and b values of Eq. (2) as it is shown by the following linear relationship:

$$R_{Mo} = -0.497 - 0.759b; \qquad r = 0.9715. \tag{3}$$

This finding indicates that the intercept, R_{Mo}, (lipophilicity) and slope, b, (specific hydrophobic surface area) for the majority of these compounds are high correlated and, in that case, they might form a homologous series of compounds as has been suggested by some authors [18–23]. Moreover, a high correlation was obtained between R_{Mo} values and the scores of the same compounds on the first principal component as it is described by the linear Eq. (4).

$$R_{Mo} = 3.985 - 2.717\text{PC1}; \qquad r = -0.9336 \tag{4}$$

The correlation of RPTLC retention parameters with other molecular descriptors calculated by the usual software packages has been also computed. The partition coefficient ($\log P$) and molar refractivity (MR) were calculated for all

compounds by means of the ChemDraw Pro. The other descriptors: surface area, volume, ovality, dipole moment (DM) and total sum of absolute charges (TAC), respectively, formed the output of SciLog *P* option of the molecular modeling computer programs ALCHEMY 2000. The values obtained are presented in Table 3 and the correlation results are showed in Table 4.

An examination of the data presented in Table 4 reveals that the sum of absolute charges is the most significant contributory factor for the lipophilicity, besides to other factors, viz. surface area, volume and ovality and with a lower contribution the DM and MR.

On the basis of these findings and as can been seen from data provided in Tables 2 and 4, the scores on the first principal component can replace efficiently the R_{Mo} values in the estimation experiments of the lipophilicity of these compounds directly from RPTLC data or via molecular descriptors. In addition, as it is shown in Fig. 2, scores plots are very useful as a display tool for examining the relationships between compounds, looking for trends, groupings or outliers. Hence,

Table 3
Computed descriptors for the benzimidazole and benztriazole derivatives studied in this paper

Compound	Area	Volume	Ovality	DM	Sum of charge[a]	MR[b]	log *P*
1	276	229	1.52	2.73	3.30	72.24	2.25
2	298	245	1.57	2.88	3.34	77.28	2.74
3	301	243	1.57	2.68	3.18	79.57	2.29
4	290	242	1.54	4.24	3.84	77.05	2.81
5	281	233	1.53	5.03	3.59	72.46	2.41
6	255	210	1.49	2.42	2.55	68.96	2.61
7	276	227	1.53	2.46	2.60	74.00	3.10
8	278	230	1.53	2.56	2.44	76.28	2.64
9	279	231	1.53	2.20	2.45	76.58	3.44
10	273	225	1.52	3.18	3.11	72.84	2.66
11	286	239	1.54	4.08	3.20	77.64	3.22
12	277	229	1.53	4.73	3.40	73.05	2.82
13	251	206	1.49	5.49	2.36	69.55	3.02
14	273	223	1.53	5.49	2.41	74.59	3.50
15	275	226	1.53	5.50	2.25	76.88	3.05
16	276	228	1.53	5.53	2.26	77.18	3.85

[a] Total sum of absolute charge.
[b] MR.

Table 4
Table of correlation of retention data and the calculated descriptors

	R_{Mo}	*b*	PC1	log *P*	MR	DM	TAC	Area	Volume	Oval
R_{Mo}	1.00	−0.97	−0.93	−0.60	0.05	−0.43	0.76	0.54	0.53	0.46
B		1.00	−0.97	0.64	0.02	0.37	−0.84	−0.49	−0.50	−0.41
PC1			1.00	0.48	−0.20	0.41	−0.59	−0.59	−0.56	−0.52
log *P*				1.00	0.22	0.42	−0.58	−0.20	−0.19	−0.09
MR					1.00	−0.06	0.10	0.83	0.81	0.85
DM						1.00	−0.12	−0.23	−0.22	−0.17
TAC							1.00	0.56	0.62	0.42
Area								1.00	0.98	0.97
Volume									1.00	0.93
Oval										1.00

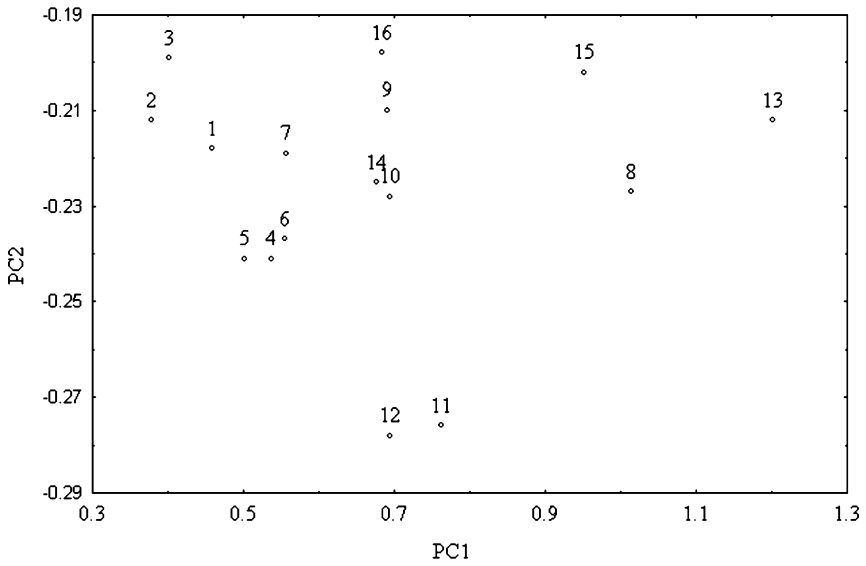

Fig. 2. Congeneric lipophilicity chart obtained by plotting scores corresponding to PC1 and PC2.

graphing scores onto the plane described by PC1 and PC2 we obtain 'the congeneric lipophilicity chart'. It appears clearly that the compounds studied in this paper form a very heterogeneous series of compounds in a good agreement with their chemical structure. Finally, a better correlation was observed between slopes and the partition coefficients ($\log P$) and other descriptors calculated for these compounds and the benzimidazole derivatives appeared the most lipophilic.

5. Conclusions

The lipophilic character of some benzimidazole and benztriazole derivatives was studied by means of reversed phase HPTLC chromatography using a mixture of methanol–water as the solvent system. The significant correlation between the R_{Mo} values and b-slopes (specific hydrophobic surface areas) indicate that this group of compounds could be considered as a homologous series independently of their structural heterogeneity as so far it was considered. The reliability of the factor scores values as lipophilicity indices is shown by their high correlation with the classical R_{Mo} values. In addition, the 'lipophilicity chart' described by the first two components had the effect of separating compounds from each other most effectively from the congeneric aspect point of view. It appeared clearly that the compounds studied in this paper form a very heterogeneous class in a very good agreement with their chemical structure. Finally, a better correlation was observed between slopes and the partition coefficients ($\log P$) and other descriptors calculated for these compounds and the benzimidazole derivatives appeared the most lipophilic.

References

[1] T.M. Evans, J.M. Gardiner, N. Mahmood, M. Smis, Bioorg. Med. Chem. Lett. 7 (1997) 409–412.
[2] I. Yalcin, B.K. Kaymakcioglu, I. Oren, E. Sener, O. Temiz, A. Akin, N. Altanlar, Il Farmaco 52 (1997) 685–691.
[3] M. Tuncbilek, H. Goker, R. Ertan, R. Eryigit, E. Kendi, N. Altanlar, Arch. Pharm. 330 (1997) 372–379.
[4] L. Garuti, M. Roberti, G. Gentilomi, Il Farmaco 55 (2000) 35–39.
[5] M.L. Lopez-Rodriguez, B. Benhamu, A. Viso, M.J. Morcillo, M. Murcia, L. Orensanz, M.J. Alfaro, M.I. Martin, Bioorg. Med. Chem. 7 (1999) 2271–2281.
[6] D. Vlaović, B. Milić, K. Mackenzie, J. Chem. Res. M (1989) 1201–1218.

[7] D.j. Vlaović, J. Čanadanović-Brunet, J. Balaž, I. Juranić, D. Djoković, K. Mackenzie, Biosci. Biotech. Biochem. 56 (1992) 199–206.
[8] N.U. Perišic-Janjic, M.M. Aćanski, N.J. Janjić, M.D. Lazarević, V. Dimova, J. Planar Chromatogr. 13 (2000) 281–284.
[9] A. Leo, C. Hansch, D. Elkins, Chem. Rev. 71 (1971) 525–616.
[10] W.J. Lyman, W.F. Reehl, D.H. Rosenblatt, Handbook of Chemical Property Estmation Methods, McGraw-Hill, New York, 1982.
[11] R.F. Rekker, R. Mannhold, in: Calculation of Drug Lipophilicity, Weinheim, 1992.
[12] A. Chiriac, D. Ciubotariu, Z. Simon, QSAR—Quantitative Relationships, Mirton Publishing House, Timisoara, 1996.
[13] R. Kaliszan, Anal. Chem. 64 (1992) 619–631.
[14] L.-G. Danielsson, Y.-H. Zhang, Trends Anal. Chem. 15 (1996) 189–198.
[15] R. Kaliszan, Trends Anal. Chem. 18 (1999) 401–410.
[16] Q.S. Wang, L. Zhang, J. Liquid Chromatogr. Rel. Technol. 22 (1999) 1–14.
[17] M.C. Garcia Alvarez-Coque, J.R. Torres Lapasió, Trends Anal. Chem. 18 (1999) 533–543.
[18] C. Horváth, W. Melander, I. Molnar, J. Chromatogr. 125 (1976) 129–156.
[19] T. Cserháti, Chromatographia 18 (1984) 18–20.
[20] G.L. Biagi, A.M. Barbaro, A. Sapone, M. Recanatini, J. Chromatogr. A 625 (1992) 392–396.
[21] G.L. Biagi, A.M. Barbaro, A. Sapone, M. Recanatini, J. Chromatogr. A 622 (1994) 341–361.
[22] G.L. Biagi, A.M. Barbaro, A. Sapone, M. Recanatini, J. Chromatogr. A 669 (1994) 246–253.
[23] F. Murakami, J. Chromatogr. 178 (1979) 393–399.
[24] S. Wold, K. Esbensen, P. Geladi, Chemom. Intell. Lab. Syst. 2 (1987) 37–52.
[25] A. Mackiewicz, W. Ratajczak, Comput. Geosci. 19 (1993) 303–342.
[26] C. Sârbu, S. Todor, J. Planar Chromatogr. 11 (1998) 123–126.
[27] C. Sârbu, S. Todor, J. Chromatogr. 822 (1998) 263–269.
[28] M. Rusu, D. Rusu, C. Sârbu, Anal. Lett. 32 (1999) 2999–3011.
[29] C. Sârbu, B. Malawska, J. Liquid Chromatogr. Rel. Technol. 23 (2000) 2143–2154.
[30] C. Sârbu, A. Mavroudis Demertzis, D. Kovala-Demertzi, Acta Chromatogr. 10 (2000) 222–229.
[31] C. Sârbu, K. Kuhajda, S. Kevrešan, J. Chromatogr. A 917 (2001) 361–366.
[32] M. Lazarevic, L.J. Klisareva, Bull. Pharm. Fac Skopje 1 (1983) 43–47.