

Relationships between structure, retention and biological activity of some Schiff base ligands and their complexes

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ABSTRACT: The lipophilicity of a series of Schiff base ligands and their complexes with nickel(II) and copper(II) has been determined by reversed-phase thin-layer chromatography using binary dioxane–water mobile phase. Chelate ligands were prepared by condensation of diamine and the corresponding β -diketone. Copper(II) and nickel(II) complexes with chelate ligands containing ethane-1,2-diamine or propane-1,2-diamine as the amine part and pentane-2,4-dione and/or 1-phenylbutane-1,3-dione, pentane-2,4-dione and/or 1,1,1-trifluoropentane-2,4-dione, or 1,1,1-trifluoropentane-2,4-dione and/or 1-phenylbutane-1,3-dione as the β -diketone part were synthesized. Some of investigated compounds were screened for their *in vitro* antifungal activity against *Sacharomyces cerevisiae* and antibacterial activity against *Escherichia coli*. Chromatographically obtained lipophilicity parameters were correlated both with calculated *n*-octanol–water partition coefficient $C \log P$ and antimicrobial activities. Satisfactory correlations were obtained. Chromatographic data proved to be reliable parameters for describing the lipophilic properties of the investigated compounds. Additionally, the principal components analysis was performed on the data chromatographically obtained. This statistical method was useful for distinguishing compounds and objective comparison of their lipophilicity parameters. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: lipophilicity; reversed-phase thin-layer chromatography; structure–retention relationship; structure–activity relationship; antimicrobial activity

INTRODUCTION

Lipophilicity is an important physicochemical property of bioactive compounds, because it is related to the tendency of molecule to be transported through biological membranes. The hydrophobic nature of a drug can be represented by a rather simple term, the logarithm of the *n*-octanol–water partition coefficient, $\log P$. Instead of the classical method for $\log P$ measurement, partition chromatographic data can be employed. Reversed-phase thin-layer chromatography (RP-TLC) is frequently used to estimate the lipophilicity of organic compounds (Slawic and Paw, 2003; Kostecka *et al.*, 2005).

It is considered that the same basic intermolecular actions determine the behavior of chemical compounds in both biological and chromatographic environments. The quantitative structure–retention relationship (QSRR) describes how the molecular structure of the investi-

gated compounds affects their chromatographic retention (Sarbu *et al.*, 2004). In the case of thin-layer chromatography, these studies are usually based on the use of R_M values defined by Bate-Smith and Westall (1950), equation (1):

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

Linear correlation between R_M values and the concentration of the organic modifier in the mobile phase (ϕ) can be expressed by equation (2):

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

where R_M^0 is lipophilicity parameter (Soczewinski and Wachtmeister, 1962) and m is the slope and represents the hydrophobic surface area (Biagi *et al.*, 1994). Equations (1) and (2) can be considered as the basis for deriving data for QSRR studies (Perišić-Janjić *et al.*, 2004; Sarbu *et al.*, 2004; Bajda *et al.*, 2007; Lazewska *et al.*, 2007).

Values of R_M^0 and m were used to calculate lipophilicity parameter C_0 (Bieganowska *et al.*, 1995) by equation (3):

$$C_0 = -R_M^0/m \quad (3)$$

The retention behavior of the solute is in relation to its chemical structure. On the other hand, the biological

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Abbreviations used: MIC, minimal inhibitory concentration; PCA, principal component analysis; QSAR, quantitative structure–activity relationship; QSRR, quantitative structure–retention relationship.

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activity of the compounds investigated is also related to the molecular structure, and these correlations are known as the quantitative structure–activity relationship (QSAR) (Brzezinska and Koska, 2006; Lazewska *et al.*, 2007). Statistical methods are frequently used for the elucidation of these relationships. Principal component analysis (PCA) is a multivariate procedure that has been used for molecular lipophilicity estimations (Sarbu and Todor, 1998; Mannhold *et al.*, 1998), for QSSR studies in chromatography (Heberger and Gorgenyi, 1999), for studying structure–property relationships (Seybold, 1999) and QSAR investigations (Drew *et al.*, 1998).

The aim of the first part of our work was (i) investigation of the retention behavior of Schiff base ligands and their complexes with Cu(II) and Ni(II), (ii) estimation of the lipophilicity parameters (R_M^0 and C_0) and (iii) correlation of these parameters with calculated *Clog P* values.

Literature data point out that Schiff bases and complexes are interesting because of their properties, such as their ability to reversibly bind oxygen (Jones *et al.*, 1979), catalytic activity in hydrogenation of olefins (Olive and Olive, 1984), transfer of an amino group (Dugas and Penney, 1981), photochromic properties (Margerum and Miller, 1971), competing abilities towards some toxic metals and antibacterial and antifungicidal properties (Raman *et al.*, 2003). Moreover, the Schiff base complexes can be greatly modified by introducing different substituents (Mohamed, 2006; Singh and Varshney, 2006; Chohan *et al.*, 2006).

Owing to our interest in the biological activity of those compounds, in order to obtain better insight in the structure–activity relationships, the aim of the second part of this work was antimicrobial assay against *Sacharomyces cerevisiae* and *Escherichia coli*.

EXPERIMENTAL

Synthesis. The Schiff base ligands and their complexes with nickel(II) and copper(II) (Table 1) were prepared by procedures described earlier (Baošić *et al.*, 2003).

Chromatography. Chromatographic separations were carried out by horizontal thin-layer chromatography on silica gel RP₁₈ plates, 20 × 20 cm (Merck, Darmstadt, Germany) using a Camag horizontal HPTLC development chamber in the tank configuration. Standard solutions (5 mg/mL) of the compounds were prepared in chloroform. The plates were spotted with 1.0 µL aliquots of freshly prepared solutions of the corresponding compounds. Before development the spotted plates were equilibrated for 30 min in a chromatographic chamber saturated with mobile phase vapor. The mobile phase used was a mixture of dioxane (as organic modifier) and water. The concentration of organic modifier in the mobile phase ranged from 50 to 80% (v/v) in 5% increments. The investigated ligands and complexes were chromatographed simultaneously. All measurements were carried out at ambient temperature. After development, the spots of individual compounds were visible owing to their natural colors. Results are expressed as means from three independent measurements.

Table 1. Structures of the compounds investigated

Compound	Ligand	R	R ₁	B	Compound	Complex
(1)	H ₂ (<i>acac</i> ₂ en) ^a	CH ₃	CH ₃	CH ₂ CH ₂	(13) (25)	[M(<i>acac</i> ₂ en)] ^c
(2)	H ₂ (<i>acac phacac</i> en) ^b	CH ₃	C ₆ H ₅	CH ₂ CH ₂	(14) (26)	[M(<i>acac phacac</i> en)]
(3)	H ₂ (<i>phacac</i> ₂ en)	C ₆ H ₅	C ₆ H ₅	CH ₂ CH ₂	(15) (27)	[M(<i>phacac</i> ₂ en)]
(4)	H ₂ (<i>phacac tfacac</i> en) ^c	C ₆ H ₅	CF ₃	CH ₂ CH ₂	(16) (28)	[M(<i>phacac tfacac</i> en)]
(5)	H ₂ (<i>acac tfacac</i> en)	CH ₃	CF ₃	CH ₂ CH ₂	(17) (29)	[M(<i>acac tfacac</i> en)]
(6)	H ₂ (<i>tfacac</i> ₂ en)	CF ₃	CF ₃	CH ₂ CH ₂	(18) (30)	[M(<i>tfacac</i> ₂ en)]
(7)	H ₂ (<i>acac</i> ₂ pn) ^d	CH ₃	CH ₃	CH(CH ₃)CH ₂	(19) (31)	[M(<i>acac</i> ₂ pn)]
(8)	H ₂ (<i>acac phacac</i> pn)	CH ₃	C ₆ H ₅	CH(CH ₃)CH ₂	(20) (32)	[M(<i>acac phacac</i> pn)]
(9)	H ₂ (<i>phacac</i> ₂ pn)	C ₆ H ₅	C ₆ H ₅	CH(CH ₃)CH ₂	(21) (33)	[M(<i>phacac</i> ₂ pn)]
(10)	H ₂ (<i>phacac tfacac</i> pn)	C ₆ H ₅	CF ₃	CH(CH ₃)CH ₂	(22) (34)	[M(<i>phacac tfacac</i> pn)]
(11)	H ₂ (<i>acac tfacac</i> pn)	CH ₃	CF ₃	CH(CH ₃)CH ₂	(23) (35)	[M(<i>acac tfacac</i> pn)]
(12)	H ₂ (<i>tfacac</i> ₂ pn)	CF ₃	CF ₃	CH(CH ₃)CH ₂	(24) (36)	[M(<i>tfacac</i> ₂ pn)]

^a *acac* = pentane-2,4-dione; en = ethane-1,2-diamine.

^b *phacac* = 1-phenylbutane-1,3-dione.

^c *tfacac* = 1,1,1-trifluoropentane-2,4-dione.

^d pn = propane-1,2-diamine.

^e M = Cu(II) (13–24) or Ni(II) (25–36).

Calculations. $C \log P$ values were calculated using the software ChemDraw 8.0 (www.cambridgesoft.com). Regression analysis was performed using NCSS (Hintze, 2001, www.ncss.com). The significance level of the performed calculations was above 95%.

The same program was used for principal component analysis. PCA was performed on the chromatographic retention data obtained for both ligands and complexes, using a covariance matrix. PCA calculates an uncorrelated set of variables (factors or PCs). These factors are ordered so that the first few retain most of the variation present in all of the original variables. As a result, PCA gives two types of information, PC scores and PC loadings. The PC score is the PC value for that compound while PC loadings present the correlations between the variables and factors.

Microbiological assay. Some of investigated Cu(II) and Ni(II) complexes were screened for their *in vitro* antifungal activity against *Sacharomyces cerevisiae* (ATCC 24858) and antibacterial activity against *Escherichia coli* (ATCC 25922, Gram-negative; Collection of Microorganisms, Department of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade). The screening of the antifungal and antibacterial activity was performed by determining the minimal inhibitory concentration (MIC) by the dilution method (Andrews, 2001).

RESULTS AND DISCUSSION

Lipophilicity determination by RP-TLC

Regular retention behavior was observed, i.e. retention decreased regularly with increasing concentration of organic modifier in the mobile phase. Retention was the greatest for the most hydrophobic compounds. A linear relationship, with satisfactory correlation coefficient, was obtained between R_M values and the concentration of organic modifier in the mobile phase (ϕ), expressed by equation (2).

In order to obtain a parameter that allowed us to analyze the results independently from the composition of the mobile phase, the R_M^0 values for investigated compounds were determined. R_M^0 is the value of R_M in pure water, and therefore could reflect the dependence of the hydrophobic properties of a compound on its structure. The most important data obtained from the plots are listed in Table 2.

High correlation was obtained between lipophilicity parameter (R_M^0) and specific hydrophobic surface area (m) as described by the linear equation (4):

$$R_M^0 = -0.418(\pm 0.180) + 0.785(\pm 0.042)m \quad (4)$$

where $r = 0.954$, $s = 0.255$, $F = 345.046$ and $n = 36$.

The correlation coefficient indicates that the substances investigated could be regarded as a homologous series independently of their structural heterogeneity (Sarbu *et al.*, 2002).

The lowest R_M^0 values were observed for compound 1. The higher R_M^0 values indicate greater lipophilicity.

Also, the lipophilicity values were lower in the case of ligands compared with lipophilicity values of their metal complexes, both with Cu(II) and Ni(II). The process of chelation of Schiff base reduces the polarity and increases the lipophilic nature of the complexes. This could be the reason for the higher R_M^0 values in complexes.

As can be seen from the data presented in Table 2, both $C \log P$ and lipophilicity values (R_M^0 and C_0) increased on substitution of CH_3 by C_6H_5 and CF_3 groups, respectively. The same trend was observed in the case of CF_3 substituted by C_6H_5 group. R_M^0 values for all investigated compounds increased with increasing number of C_6H_5 groups. Substitution also substantially affected the slope values (measurement of the hydrophobic surface area). With the increasing number of C_6H_5 groups, the hydrophobic surface area of the compounds increased (Table 2).

Further, the results obtained throughout this study clearly showed that the lipophilicity of the Schiff base ligands and complexes increased on substitution of a low polarity CH_3 group by a more polar CF_3 group. These results are in accordance with previous reports of the lipophilicity estimation of some derivatives of (2-thio)hydantoin (Lazewska *et al.*, 2007). The results indicated that lipophilic character is not only affected by the composition and structure of the molecule and that some other interactions could be important. It can be assumed that the electron-withdrawing properties and strong resonance of CF_3 and C_6H_5 result in a substantial increase in the lipophilicity. Substitution of an ethane-1,2-diamine with propane-1,2-diamine also resulted in increase of lipophilicity values. These results are in accordance with previous reports of the retention behavior of some tris- β -diketonato complexes and aromatic carboxamides (Hollosoy *et al.*, 2002; Natić *et al.*, 2005).

Relationship between chromatographically obtained and calculated parameters of lipophilicity

Calculated $C \log P$ values (Table 2) were compared with the lipophilicity values, R_M^0 and C_0 , evaluated from the chromatographic results. Satisfactory correlations were obtained. The obtained relationships are described by the equations (5)–(10).

Ligands (1–12):

$$R_M^0 = 1.246(\pm 0.350) + 0.517(\pm 0.150)C \log P \quad (5)$$

where $r = 0.737$, $s = 0.408$, $F = 11.907$ and $n = 12$.

$$C_0 = 0.460(\pm 0.080) + 0.096(\pm 0.034)C \log P \quad (6)$$

where $r = 0.664$, $s = 0.093$, $F = 7.899$ and $n = 12$.

Complexes of Cu(II) (13–24):

$$R_M^0 = -0.390(\pm 0.414) + 0.772(\pm 0.092)C \log P \quad (7)$$

where $r = 0.936$, $s = 0.365$, $F = 71.208$ and $n = 12$.

Table 2. Lipophilicity parameters (R_M^0 , C_0 , $C \log P$), regression data and scores on the principal components for compounds studied

Compound	R_M^0	$-m$	$-r$	s	F	C_0	$C \log P$	PC1	PC2
(1)	1.579 (± 0.121)	3.121 (± 0.183)	0.991	0.049	289.786	0.506	0.808	-2.127	-1.359
(2)	2.464 (± 0.081)	4.093 (± 0.124)	0.998	0.033	1092.246	0.602	1.998	-1.199	0.153
(3)	3.438 (± 0.136)	5.129 (± 0.207)	0.996	0.055	613.135	0.670	3.188	-0.145	1.193
(4)	2.050 (± 0.189)	2.314 (± 0.279)	0.972	0.058	69.112	0.886	2.662	1.386	-2.154
(5)	2.469 (± 0.102)	4.071 (± 0.155)	0.996	0.041	691.865	0.606	1.475	-1.095	0.198
(6)	2.158 (± 0.203)	2.821 (± 0.309)	0.971	0.082	83.570	0.765	2.136	0.691	-1.818
(7)	1.719 (± 0.175)	3.271 (± 0.266)	0.984	0.070	151.127	0.525	1.117	-1.950	-1.118
(8)	2.445 (± 0.135)	4.007 (± 0.206)	0.993	0.054	379.823	0.610	2.307	-1.033	0.139
(9)	3.465 (± 0.121)	5.136 (± 0.184)	0.997	0.049	778.790	0.675	3.497	-0.065	1.080
(10)	2.164 (± 0.194)	2.621 (± 0.296)	0.970	0.078	78.683	0.826	2.971	1.175	-2.481
(11)	2.455 (± 0.199)	4.021 (± 0.303)	0.986	0.080	176.584	0.611	1.781	-1.018	0.196
(12)	2.188 (± 0.097)	2.836 (± 0.148)	0.993	0.039	366.362	0.772	2.445	0.794	-1.660
(13)	1.715 (± 0.100)	2.857 (± 0.147)	0.995	0.031	376.884	0.600	2.223	-1.178	-0.230
(14)	3.009 (± 0.210)	4.371 (± 0.308)	0.990	0.064	200.878	0.688	3.926	0.181	0.906
(15)	4.308 (± 0.230)	5.811 (± 0.338)	0.993	0.071	295.596	0.741	5.629	1.463	-0.354
(16)	3.509 (± 0.256)	4.840 (± 0.377)	0.988	0.079	165.213	0.725	5.067	0.728	0.010
(17)	2.134 (± 0.094)	3.411 (± 0.138)	0.997	0.029	612.036	0.626	3.364	-0.508	1.116
(18)	2.679 (± 0.046)	4.040 (± 0.068)	0.999	0.146	3511.820	0.664	4.504	-0.221	1.289
(19)	1.632 (± 0.138)	2.657 (± 0.203)	0.989	0.042	171.834	0.614	2.742	-0.961	0.340
(20)	3.092 (± 0.194)	4.491 (± 0.285)	0.992	0.060	248.011	0.688	4.445	0.330	0.866
(21)	4.608 (± 0.260)	6.194 (± 0.382)	0.992	0.080	263.505	0.744	6.148	1.504	-0.267
(22)	4.146 (± 0.294)	5.669 (± 0.432)	0.989	0.090	172.522	0.731	5.586	0.842	0.617
(23)	2.304 (± 0.171)	3.520 (± 0.262)	0.992	0.041	180.093	0.655	3.882	-0.192	0.743
(24)	2.760 (± 0.185)	3.983 (± 0.271)	0.991	0.057	215.691	0.693	5.023	0.405	1.150
(25)	1.654 (± 0.111)	2.907 (± 0.169)	0.992	0.044	296.756	0.569	2.223	-1.414	-1.208
(26)	3.176 (± 0.098)	4.643 (± 0.149)	0.997	0.039	144.105	0.684	3.926	0.080	0.711
(27)	3.933 (± 0.246)	5.207 (± 0.374)	0.987	0.099	193.350	0.755	5.629	1.293	0.146
(28)	3.874 (± 0.178)	5.271 (± 0.270)	0.993	0.071	380.126	0.735	5.067	0.958	0.488
(29)	2.905 (± 0.111)	4.436 (± 0.169)	0.996	0.045	688.891	0.655	3.364	-0.376	0.651
(30)	3.172 (± 0.247)	4.507 (± 0.375)	0.983	0.099	144.105	0.704	4.504	0.437	0.403
(31)	1.772 (± 0.075)	2.950 (± 0.115)	0.996	0.030	662.661	0.601	2.742	-1.069	-1.038
(32)	3.367 (± 0.063)	4.921 (± 0.096)	0.999	0.026	2605.494	0.684	4.445	0.106	0.831
(33)	3.785 (± 0.244)	4.986 (± 0.372)	0.986	0.098	179.913	0.759	6.148	1.306	-0.026
(34)	3.860 (± 0.159)	5.307 (± 0.241)	0.995	0.064	483.321	0.727	5.586	0.912	0.730
(35)	2.608 (± 0.148)	3.957 (± 0.226)	0.992	0.060	307.162	0.659	3.882	-0.389	0.096
(36)	3.014 (± 0.184)	4.279 (± 0.280)	0.989	0.074	232.897	0.704	5.023	0.352	0.341

m = slope, r = regression coefficient, s = standard error, F = value of F -test of significance.

$$C_0 = 0.506(\pm 0.016) + 0.040(\pm 0.004)C \log P \quad (8)$$

where $r = 0.962$, $s = 0.014$, $F = 124.286$ and $n = 12$.
Complexes of Ni(II) (25–36):

$$R_M^0 = 0.507(\pm 0.364) + 0.591(\pm 0.080)C \log P \quad (9)$$

where $r = 0.919$, $s = 0.320$, $F = 53.966$ and $n = 12$.

$$C_0 = 0.480(\pm 0.016) + 0.047(\pm 0.004)C \log P \quad (10)$$

where $r = 0.973$, $s = 0.014$, $F = 174.478$ and $n = 12$.

From equations (5) and (6) it can be seen that moderate correlation coefficients were obtained in the case of the studied ligands (1–12). The trifluoromethyl group present in the structure of compounds 4, 6, 10 and 12 could be the reason for the bad correlations. This is not quite unexpected since a particular behavior is observed for fluorine-substituted compounds (Reta *et al.*, 2003) and is attributed to special properties of fluor, such as high electronegativity, relatively small size and very low polarizability (Smart, 2001).

After excluding compounds 4, 6, 10 and 12 from calculations, better correlation coefficients were obtained. The correlations are expressed by equations (11)–(12):

$$R_M^0 = 1.100(\pm 0.180) + 0.695(\pm 0.082)C \log P \quad (11)$$

where $r = 0.961$, $s = 0.204$, $F = 72.586$ and $n = 8$.

$$C_0 = 0.481(\pm 0.021) + 0.059(\pm 0.009)C \log P \quad (12)$$

where $r = 0.933$, $s = 0.023$, $F = 40.399$ and $n = 8$.

No such deviation was observed in the case of Cu(II) and Ni(II) complexes, which is probably the consequence of trifluoromethyl electron delocalization over the chelate rings. The graphs obtained for these correlations are presented in Figs 1 and 2.

Principal component analysis

The main advantage of PCA in chromatography is the clustering of solutes according to their retention behavior. The scores plots are very useful as a display

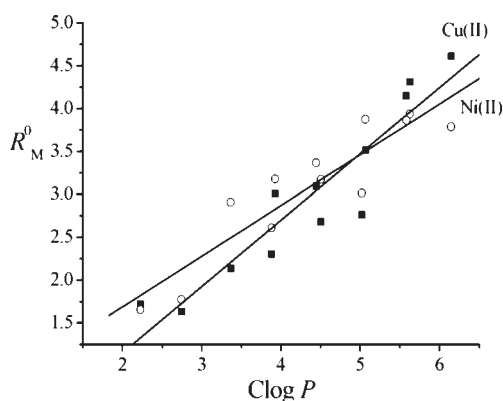


Figure 1. Correlations between R_M^0 values and calculated $C \log P$ values for Cu(II) and Ni(II) complexes.

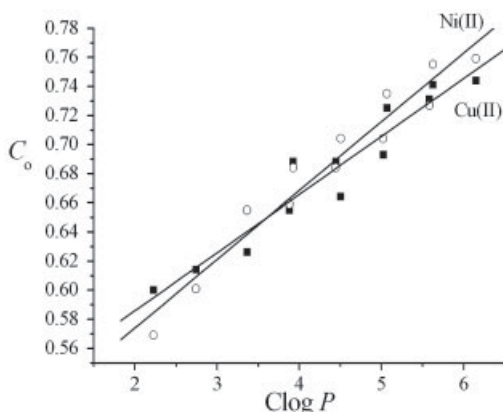


Figure 2. Correlations between C_0 values and calculated $C \log P$ values, for Cu(II) and Ni(II) complexes.

tool for examining the relationship between compounds and looking for trends, groupings and outliers. The first principal component (PC1) defines as much of the variation in the retention 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. In chromatography two or three principal components are often sufficient to describe most of the retention data variation. In this work PCA has been developed primarily to find similarities and dissimilarities between the compounds investigated and their retention.

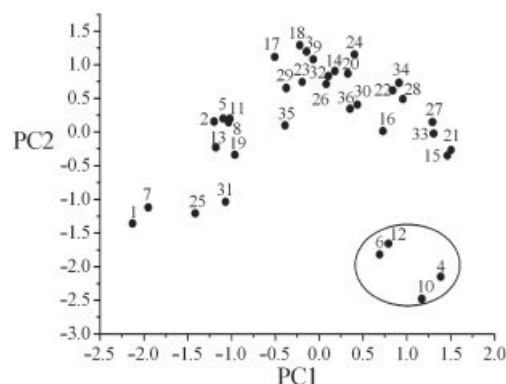


Figure 3. PC1-PC2 score plot of the retention R_F -values ('congeneric lipophilicity chart').

The results of PCA, compiled in Table 3, suggest that a two-component model explains 97.12% of the total variance. The first principal component PC1 accounted for 88.74% of data variance and the second principal component PC2 for 8.39%.

Inputs ('scores') of individual compounds to PC1 and PC2, given in Table 2, determine their distribution on the principal component plane (Fig. 3). The application of PCA reveals that ligands with CF_3 substituents (compounds **4**, **6**, **10** and **12**) form separate clusters, in agreement with their structural characteristics and their structural dissimilarity with the remaining analytes. Also, the cluster formation suggests that there is an influence of the structural characteristic of those four compounds on their retention behavior. In contrast, compounds **5** and **11** do not belong together with **4**, **6**, **10** and **12**, despite their almost identical structures. The reason for this behavior could be the presence of the methyl group with a significantly lower inductive and resonance effects in comparison to the trifluoromethyl group (Smart, 2001). As a result, trifluoromethyl electron delocalization over the whole ligand is more pronounced, as in complexes. This is in accordance with some previously reported results (Raman *et al.*, 2001).

Linear correlations (13–15) were observed between lipophilicity parameter, R_M^0 , and first principal component, PC1. From the statistical data obtained it can be concluded that the scores on the first principal

Table 3. The eigenvalues and the ratios of the variance explained by the three components using covariance matrix

No.	Eigenvalue	Individual percentage	Cumulative percentage
1	0.121126	88.74	88.74
2	0.011447	8.39	97.12
3	0.002433	1.78	98.90
4	0.000633	0.46	99.37
5	0.000446	0.33	99.69
6	0.000246	0.18	99.87
7	0.000171	0.13	100.00

Table 4. Antimicrobial activity of some of the investigated compounds

<i>Escherichia coli</i>		<i>Sacharomyces cerevisiae</i>	
Compound	MIC (mmol/L)	Compound	MIC (mmol/L)
13	8.27	13	4.13
14	3.60	14	1.81
15	5.84	19	4.14
19	8.25	20	1.74
20	3.46	25	5.29
25	5.61	31	8.79
27	4.14	33	5.97
31	7.06		

component can efficiently replace the R_M^0 values in the lipophilicity estimations directly from the RP-TLC data. Ligands (**1–12** without **4, 6, 10, 12**):

$$R_M^0 = 3.504(\pm 0.050) + 0.926(\pm 0.039)PC1 \quad (13)$$

where $r = 0.995$, $s = 0.076$, $F = 560.815$, $n = 8$.

Complexes of Cu(II) (**13–24**):

$$R_M^0 = 2.768(\pm 0.073) + 1.120(\pm 0.087)PC1 \quad (14)$$

where $r = 0.971$, $s = 0.247$, $F = 166.392$, $n = 12$.

Complexes of Ni(II) (**25–36**):

$$R_M^0 = 2.938(\pm 0.065) + 0.846(\pm 0.075)PC1 \quad (15)$$

where $r = 0.962$, $s = 0.220$, $F = 125.614$, $n = 12$.

Relationship between antimicrobial activity and lipophilicity parameters

The investigated solutes were screened for their *in vitro* antifungal and antibacterial activity against representative strains. The minimal inhibitory concentrations (MIC values in mmol/L) for the investigated solutes are presented in Table 4.

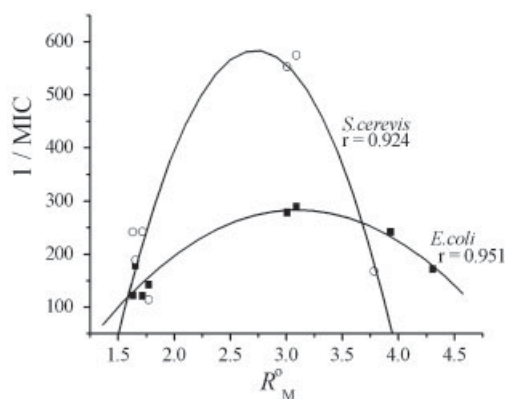
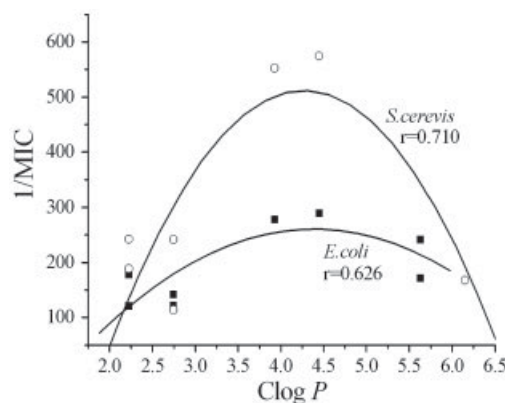
The results shown in Table 4 clearly indicate that there is a large difference in biological activity between compounds. Investigated complexes produced marked biological activity with MICs values ranging from 1.74 to 8.79 mmol/L. These results are in accordance with

chromatographically obtained lipophilicity parameters, R_M^0 , i.e. investigated compounds having higher R_M^0 values showed higher inhibitory activities (lower MIC), probably due to their easier permeation through the lipid layer of the cell membrane of a microorganism.

To evaluate the structure–activity relationship and to study the biological significance of lipophilicity, we attempted to correlate antimicrobial activity (expressed as $1/\text{MIC}$) with lipophilicity parameters (R_M^0 and $C \log P$).

The effects of the chromatographically obtained lipophilicity parameter R_M^0 on the antibacterial and antifungal response against *Escherichia coli* and *Sacharomyces cerevisiae*, respectively, are shown in Fig. 4. The parabolic dependence obtained between experimentally determined lipophilicity of the compounds and their antimicrobial activity (Fig. 4) indicates the possibility of using chromatography to choose compounds suitable for microbiological investigations. The absence of linearity points out that some other factors besides lipophilicity could be involved in the antimicrobial mode of action.

Correlations $1/\text{MIC}$ vs $C \log P$ are depicted in Fig. 5. Regression coefficients presented on both Figs 4 and 5 clearly indicate better correlation in the case of chromatographically obtained lipophilicity parameters. Therefore it could be concluded that, for this kind of study, chromatographically obtained lipophilicity contains more valuable information than calculated

**Figure 4.** Relationship between antimicrobial activity and R_M^0 values.**Figure 5.** Relationship between antimicrobial activity and $C \log P$.

$C \log P$ values. The reason could lie in the fact that the program used for lipophilicity calculations did not recognize the difference between Cu(II) and Ni(II) complexes (Table 2), giving identical $C \log P$ values.

CONCLUSIONS

Reversed-phase thin-layer chromatography was successfully used to evaluate the lipophilic parameters (R_M^0 and C_0) of some Schiff base ligands and their Cu(II) and Ni(II) complexes. The chromatographic lipophilicities obtained were compared both with calculated $C \log P$ values and antimicrobial activities. Satisfactory correlations were obtained.

The presented study revealed that the chromatographically obtained lipophilicity contains more valuable information than calculated values and can be used for further quantitative structure–activity relationship studies of these compounds. Lipophilicity determined in this way for a group of derivatives helps to explain the differences between the microbiological activities of compounds of similar structure. Moreover, in an attempt to generate novel structures with interesting biological activities, this study could provide useful information as the basis for designing new molecules with the desired properties.

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