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Relationship between structure and reversed-phase thin-layer chromatographic lipophilicity parameters in a group of piperazine derivatives

Petr Kastner[a,*], Miroslav Kuchař[b], Jiří Klimeš[a], Dana Dosedlová[b]

[a]*Pharmaceutical Faculty, Charles University, Department of Pharmaceutical Chemistry and Drug Control, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic*

[b]*VÚFB, a.s., Research Institute of Pharmacy and Biochemistry, Kouřimská 17, 130 60 Prague 3, Czech Republic*

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Abstract

The R_M values of substituted piperazine derivatives, **I**, have been determined on silanized silica gel plates with buffered aqueous acetone with or without *n*-octylamine as the mobile phase. Chromatographically determined lipophilicity of substances containing aromatic nuclei that are separated by a sufficiently flexible chain, presented a decrease in comparison with the lipophilicity calculated on the basis of tabulated fragmental constants. The intramolecular hydrophobic interaction of aromatic nuclei resulting from their stacking conformation could be a probable explanation for this phenomenon.

Keywords: Lipophilicity; Piperazines

1. Introduction

Lipophilicity is the decisive property of bioactive compounds for their bioavailability [1] and plays an important role in the interaction of a ligand with the relevant biomacromolecule at the site of action [2,3]. The fundamental experimental lipophilic quantity is the logarithm of the partition coefficient in the reference system, octanol–water [4]. The additivity principle [5] made it possible to define [6,7] the lipophilicity parameters, π, and the fragmental constants, f. Using the fragmental constants and fragmental factors [7], the logarithm of the partition coefficient can be expressed by Eq. (1); and from the known values of fragmental constants and factors it can be calculated. The values of R_M from reversed-phase thin-layer chromatography (TLC) and the logarithms of capacity factors from high-performance liquid chromatography (HPLC) are frequently used as lipophilicity parameters. If the Collander equation [8] (Eq. (2)) holds for the chromatographic and reference system, octanol–water, the use [9–11] of chromatographic quantities is equivalent to log P according to Eq. (3).

$$\log P = \sum a_i f_i + \sum b_j F_j \quad (1)$$

$$\log P = a \cdot \log P_s + b \quad (2)$$

$$\log P = a \cdot R_M + b_1 \quad (3)$$

where P is the partition coefficient, f is fragmental

*Corresponding author.

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constant, F is a fragmental factor, a and b are constants, P_s is the partition coefficient obtained from the chromatographic system and R_M characterizes the molecular lipophilicity in RP-TLC and can be calculated as: $R_M = \log(1/R_F - 1)$. Lipophilicity of compounds containing two or more aromatic nuclei that are separated by a sufficiently flexible chain can be influenced by their intramolecular hydrophobic interaction, leading to a decrease in the whole lipophilicity [12]. We have already met with this decrease in the group of phenylalkoxyarylacetic [13] or 2-phenylalkoxyarylpropionic acids [14]. In these compounds, lower values of experimentally determined log P, in comparison with the values calculated from tabulated parameters, π, or fragmental constants, f, were found. The influence of the above-mentioned hydrophobic interaction on the retention behavior of these acids was studied [13] also in TLC on silica gel that was impregnated with silicone oil as the stationary phase. The chromatographic systems used reflect the lipophilicity changes in an analogous way to that of the reference system, octanol–water.

2. Experimental

2.1. TLC

Silanized Kieselgel 60 without a fluorescent indicator (E. Merck, Darmstadt, Germany), was used as the stationary phase. Impregnation was carried out by developing the glass plates (20×20 cm) with a 5% ethereal solution of silicone oil (DC 200). Ether was evaporated within 16 h at 22°C. A 2-μl volume of a methanolic solution (0.2%) of the compounds under study was applied to the plate 2 cm from the lower edge. After evaporating the methanol at 22°C, ascending one-dimensional TLC was carried out using 5% ammonium hydroxide (pH 11.3)–acetone (40:60, v/v) with or without 1% *n*-octylamine as the mobile phase. The normal chromatographic chamber (22×22×6.5 cm) containing 100 ml of eluent was lined with chromatographic paper. The equilibration time to saturate the chamber atmosphere was 2 h at 22°C. After migration for 15 cm, the plates were removed. The remaining mobile phase was evaporated off and the compounds were detected under UV light at 366 nm and then with iodine vapour as brown spots. Each TLC measurement was replicated three times, samples were applied to the thin-layer plate in a random sequence. Each chromatogram contained six compounds and two bases that served as reference samples. In the individual chromatograms, the R_F values did not differ by more than 0.02.

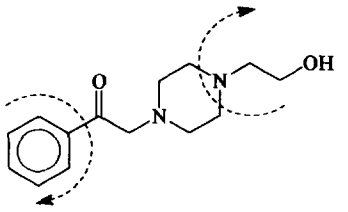
2.2. Calculations

The coefficients in the regression equations were calculated by multiple regression analysis (Statgraphics Programme, Version 4.2). The statistical significance of the regression equations was tested by the correlation coefficient (r), the standard deviation (s) and the Fischer–Snedecor criterion (F).

The calculated values of $\Delta\log P_{th}$ (P_{th} is the theoretically calculated partition coefficient) were obtained by the use of data on fragmental constants and log P values from Hansch and Leo [15]. Only the variable parts of molecules of piperazine derivatives (cf. Fig. 1) were considered. The tabulated values of fragmental constants for the aromatic ring and substituents were used for calculations in the aromatic part of the molecule. The fragmental constants for substituents, R, were calculated using the log P values of the corresponding amines. The examples on Fig. 1 illustrate the method of calculation.

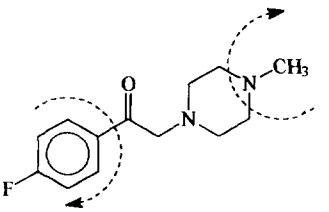
3. Results and discussion

In a group of piperazine derivatives of general formula **I**, their chromatographic behavior was evaluated by the reversed-phase method on chemically modified silica gel impregnated with silicone oil. The relationship of the chromatographic quantities, R_M to the lipophilicity of the compounds under study was investigated by means of regression equations expressing the dependence of the R_M values on the lipophilic parameters $\Delta\log P_{th}$, calculated for the variable parts of the molecules (see Section 2.2).



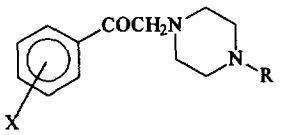
$\Delta \log P_{th} = f(C_6H_5) + f(HOCH_2CH_2N<) = 1.90 + \log P\,(HOCH_2CH_2NH_2) - 2f(H) =$

$= 1.90 - 1.31 - 0.46 = 0.13$



$\Delta \log P_{th} = f(F) + f(C_6H_4) + \log P\,(CH_3NH_2) - 2f(H) = 0.37 + 1.67 - 0.57 - 0.46 = 1.01$

Fig. 1. Examples of $\Delta\log P_{th}$ calculations.



I

As the compounds under study are of basic character, their possible interactions with free silanol groups was limited by the addition of *n*-octylamine to the mobile phase. The values of chromatographic quantities and lipophilic parameters are summed up in Table 1. For the sake of comparison, the chromatography of compounds **I**, was carried out also using the mobile phase without any addition of *n*-octylamine. A stacking conformation of both aromatic nuclei, making their intramolecular hydrophobic interaction possible, was assumed in substances **I**, in which the second substituent on the nitrogen atom *R* is a benzyl or a substituted benzyl. Therefore, only the R_M and $\Delta\log P_{th}$ values of substances **Ia** to **Ik**, in which the above-mentioned interaction cannot take place, were used to derive regression Eqs. (4,5). Eq. (4) makes use of the R_M values in the mobile phase without octylamine and Eq. (5) makes use of the R_M values in the mobile phase with octylamine.

		n	*r*	*s*	*F*
$\Delta \log P_{th} = 3.122R_M + 2.149$	(4)	10	0.984	0.201	242.0
$\Delta \log P_{th} = 3.235R_M + 1.933$	(5)	10	0.985	0.191	269.8

It is clear from the equations that the addition of octylamine to the mobile phase does not decide the statistical significance of both regression relationships. Thus, the chromatographic behavior probably is not influenced by the interaction of compounds with free silanol groups.

For the remaining derivatives with **II** to **Is**, the pertinent lipophilic parameters, $\Delta\log P_{exp}$, were calculated from the experimental R_M values by inserting them into Eqs. (4,5). The results are summed up in Table 2.

It is evident that, for these compounds, their lipohilicity, determined on the basis of the chromatographic quantities, is lower than the value corresponding to the tabulated values of fragmental constants. The mean value of a decrease in lipohilici-

Table 1
Chromatographic and lipophilic quantities of aryloxoethylderivatives, I (series A)

Number	X	R	R_F[a]		R_M[a]		Δlog P_{th}[b]	Δlog P_{calc}[c]	
			(1)	(2)	(1)	(2)		Eq. (6)	Eq. (7)
Ia	H	H	0.73	0.70	−0.43	−0.37	0.43	0.81	0.73
Ib	H	$COOC_2H_5$	0.63	0.58	−0.23	−0.14	1.21	1.43	1.47
Ic	H	CH_2CH_2OH	0.85	0.80	−0.75	−0.60	0.13	−0.21	−0.01
Id	H	CH_3	0.73	0.70	−0.43	−0.37	0.87	0.81	0.73
Ie	H	C_6H_5	0.48	0.41	0.03	0.16	2.34	2.26	2.45
If	4-C_6H_5S	CH_2CH_2OH	0.62	0.59	−0.22	−0.16	1.47	1.47	1.41
Ig	4-C_6H_5S	CH_3	0.49	0.45	0.02	0.08	2.21	2.23	2.19
Ih	4-C_6H_5S	C_6H_5	0.26	0.225	0.45	0.54	3.68	3.60	3.67
Ii	2-C_6H_5S	CH_3	0.49	0.46	0.02	0.06	2.21	2.23	2.12
Ik	2-(4-$CH_3OC_6H_4$)S	CH_3	0.49	0.50	0.02	0	2.19	2.23	1.93
Il	H	$C_6H_5CH_2$	0.53	0.48	−0.05	0.04	2.53		
							(1.92)	2.01	2.06
Im	H	4-$ClC_6H_4CH_2$	0.44	0.39	0.10	0.19	3.24		
							(2.63)	2.48	2.54
In	4-C_6H_5S	$C_6H_5CH_2$	0.32	0.265	0.33	0.43	3.87		
							(3.26)	3.21	3.32
Io	2-C_6H_5S	$C_6H_5CH_2$	0.315	0.265	0.34	0.43	3.87		
							(3.26)	3.25	3.32
Ip	2-(4-$CH_3OC_6H_4$)S	$C_6H_5CH_2$	0.315	0.30	0.34	0.37	3.85		
							(3.24)	3.25	3.12

[a] (1) Without *n*-octylamine in the mobile phase and (2) with *n*-octylamine in the mobile phase.
[b] Values were calculated for the variable part of molecules according to the examples in Section 2; corrected values for benzyl derivatives are shown in brackets (the value, 0.61, was subtracted).
[c] Values calculated from Eqs. (6,7), respectively, by introducing R_M values.

ty expressed by means of $\Delta f_{|H|}$ calculated from all ten values quoted in Table 2 is −0.61. We suppose that this decrease results from the folding conformation, which enables both aromatic nuclei to approach each other, allowing their mutual hydrophobic interaction.

Table 2
Calculated values of Δlog *P* for benzyl derivatives **II–Ip**

Number	Δlog P_{th}	Δlog P_{exp}			Δf[c]
		(1)[a]	(2)[b]	∅	
Il	2.53	1.98	2.06	2.02	−0.51
Im	3.24	2.44	2.56	2.50	−0.74
In	3.87	3.16	3.41	3.28	−0.59
Io	3.87	3.21	3.33	3.27	−0.60
Ip	3.85	3.21	3.13	3.25	−0.60

[a] Calculated from R_M values (1) by introducing the result into Eq. (4).
[b] Calculated from R_M values (2) by introducing the result into Eq. (5).
[c] Correction for hydrophobic interaction, calculated as the difference between the average value of Δlog P_{exp} and Δlog P_{th}, the average correction $\Delta f_{|H|} = -0.61$.

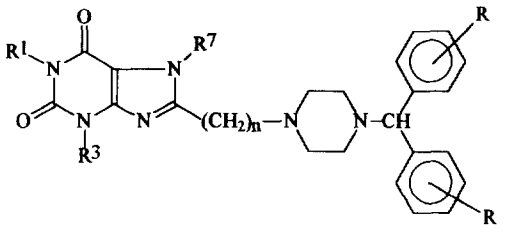
If the correction for the hydrophobic interaction (in the value $\Delta f_{|H|} = -0.61$) is considered in the calculation of the Δlog P_{th} values of benzyl derivatives **II** to **Is**, regression Eqs. (6,7) are obtained. Eq. (6) is derived for the mobile phase without octylamine and Eq. (7) is for the mobile phase with octylamine.

		n	*r*	*s*	*F*
$\Delta \log P_{th} = 3.174R_M + 2.167$	(6)	15	0.989	0.166	574.4
$\Delta \log P_{th} = 3.234R_M + 1.928$	(7)	15	0.990	0.161	610.1
$\Delta \log P_{th} = 3.644R_M + 2.385$	(8)	15	0.968	0.326	196.6
$\Delta \log P_{th} = 3.731R_M + 2.109$	(9)	15	0.974	0.296	240.9

The statistical criteria of these equations confirm the influence of a decrease in lipophilicity on the chromatographic behavior of the benzyl derivatives under study. For the sake of comparison, the corresponding regression Eqs. (8,9) were derived, in which the lipophilicity of the benzyl derivatives was considered without correction for the intramolecular hydropho-

bic interaction. The statistical significance of these equations was markedly decreased.

The existence of a folded conformation in piperazine derivatives, **I**, is supported by the conformational analysis of xanthine derivatives with a piperazine moiety of general formula, **II**, as carried out by Walther et al. [16].



II

Using the SYBYL software (Tripos, St. Louis, USA), they found that in the case of the connection of xanthine and piperazine rings by one carbon atom, the flexibility of the molecule is markedly limited and the extended conformation is thus preserved. If the connecting chain is extended to 2–3 atoms, in substances **II**, 20–30 low energy conformations, with an energy range of 2 kcal/mol, are found. Such small energy differences result from the high flexibility of the carbon chain, indicating that in such cases both non-compact and compact conformations exist.

With the restriction of an oxygen atom, instead of one carbon atom, we can apply the above-mentioned results to our piperazine derivatives. From the viewpoint of energy, the hydrophobic intramolecular interaction can thus be considered acceptable.

The authorization of the use of mentioned correction of a decrease in lipophilicity was confirmed also with its use in a slightly modified series of derivatives **I**. The results of chromatographic evaluation of this series are summed up in Table 3. Eq. (10) was derived for the relationship between $\Delta\log P_{th}$ values and the corresponding R_M values for the whole series in which the corrected values for benzyl derivatives **II**, **Im** and **Iu–Iz** were used. The calculated values, $\Delta\log P_{calc}$ (last column in Table 3) are in good agreement with the corrected values $\Delta\log P_{th}$.

		n	r	s	F
$\Delta\log P_{th} = 3.224R_M + 1.895$	(10)	16	0.984	0.191	434.1

In conclusion, it can be stated that the retention behaviors of the derivatives of piperazine **I** under study are obviously determined by their lipophilicity. Retention behavior is probably influenced by the intramolecular hydrophobic interaction of an aromatic nuclei that is separated by a sufficiently long chain, making possible their stacking conformation.

Table 3
Chromatographic and lipophilic quantities of aryloxoethylderivatives **I** (series B)

Number	X	R	R_F	R_M	$\Delta\log P^{a}_{th}$	$\Delta\log P^{b}_{calc}$
Ia	H	H	0.69	−0.35	0.43	0.77
Ib	H	$COOC_2H_5$	0.57	−0.12	1.21	1.51
Ic	H	CH_2CH_2OH	0.815	−0.64	0.13	−0.17
Id	H	CH_3	0.69	−0.35	0.87	0.77
Ie	H	C_6H_5	0.42	0.14	2.34	2.35
Ir	4-F	CH_2CH_2OH	0.775	−0.54	0.27	0.15
Is	4-F	CH_3	0.64	−0.25	1.01	1.09
It	2,4-Cl_2	$COOC_2H_5$	0.39	0.19	2.63	2.51
Il	H	$C_6H_5CH_2$	0.50	0	1.92	1.90
Im	H	4-$ClC_6H_4CH_2$	0.38	0.21	2.63	2.57
Iu	H	4-i-$C_3H_7C_6H_4CH_2$	0.31	0.36	3.32	3.06
Iv	4-F	$C_6H_5CH_2$	0.46	0.08	2.06	2.15
Iw	4-Cl	$C_6H_5CH_2$	0.35	0.27	2.63	2.77
Ix	2,4-Cl_2	$C_6H_5CH_2$	0.28	0.41	3.34	3.22
Iy	4-CH_3	$C_6H_5CH_2$	0.42	0.14	2.42	2.35
Iz	4-OCH_3	$C_6H_5CH_2$	0.425	0.13	2.08	2.31

[a] cf. note [b] below Table 1.
[b] Calculated by inserting the R_M values into Eq. (10).

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