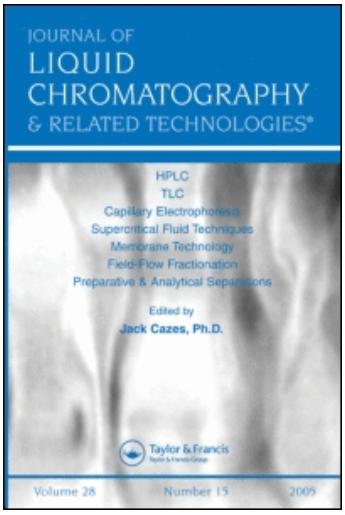

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Beata Morak[a]; Małgorzata Nowak[a]; Krystian Pluta[a]
[a] Department of Organic Chemistry, The Medical University of Silesia, Sosnowiec, Poland

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Determination of the Lipophilicity Parameters R_{M0} and Log P of New Azaphenothiazines by Reversed-Phase Thin-Layer Chromatography†

Beata Morak, Małgorzata Nowak, and Krystian Pluta
Department of Organic Chemistry, The Medical University of Silesia, Sosnowiec, Poland

Abstract: The lipophilicity parameters (R_{M0} and $\log P_{TLC}$) of three types of azaphenothiazines **1**–**3** were determined by reversed-phase thin-layer chromatography on RP-18 silica plates with acetone-aqueous TRIS (tris(hydroxymethyl)aminomethane) buffer as the mobile phase. The R_M values were linearly dependent on the concentration of acetone, and extrapolated to 0% of acetone, gave the lipophilicity parameter R_{M0}. The parameter R_{M0} and specific hydrophobic surface area b were significantly intercorrelated showing a congeneric class of azaphenothiazines **1**–**3**. The parameter $\log P_{TLC}$ was determined from the R_{M0} values by use of a calibration curve obtained for five standards. The determined parameters were discussed in the terms of structure lipophilicity relationships and compared with data obtained from seven calculation programs.

Keywords: Lipophilicity parameters, R_{M0}, Log P, Azaphenothiazines, Reversed-phase, TLC

INTRODUCTION

Lipophilicity is a very important molecular property used in QSAR studies and plays a crucial role in the design of new drugs with required biological activity. Lipophilicity is expressed by the logarithm of the partition coefficient, $\log P$, determined in the reference system of n-octanol-water. The

†Part XCI in the series of Azinyl Sulfides.

Address correspondence to Krystian Pluta, Department of Organic Chemistry, The Medical University of Silesia, Jagiellońska 4, 41-200, Sosnowiec, Poland. E-mail: pluta@slam.katowice.pl

traditional method of determination of log P, 'shake flask', is troublesome and limited (not very suitable for compounds with $\log P > 3$).[1,2] Therefore, this method is replaced by other experimental methods, most often chromatographic ones (reversed-phase thin-layer chromatography RP TLC[3–5] and reversed-phase high performance chromatography RP HPLC).[6] Moreover, the R_{M0} values obtained from RP TLC (by extrapolation of the R_M values to zero concentration of an organic modifier) are widely used as a chromatographic alternative parameter to the log P values (describing partitioning between non-polar stationary and polar mobile phases),[3] or are calculated to the log P_{TLC} values using a calibration curve with standards of known lipophilicity ($\log P_{lit.}$).[5]

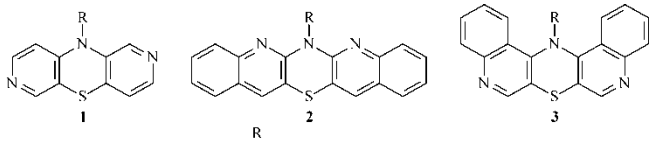
Phenotiazines form a significant class of heterocyclic compounds having wide chemical properties and very interesting biological activities (antipsychotic and anticancer). Some modifications of the phenothiazine structures were directed into azaphenothiazines, where the benzene ring was substituted with an azine ring.[7] In continuation of our search for pharmacoactive pyridine and quinoline derivatives, we modified the phenothiazine structure with the pyridine and quinoline ring to obtain tricyclic and pentacyclic azaphenothiazines **1**–**3** (Scheme 1) of potential antipsychotic, antidepressant, antihistaminic, antiasthmatic, anticancer, and sedative activity.[8] For phenothiazines used as neuroleptics, a good correlation between lipophilicity and selected biological actions was reported.[9,10]

The purpose of this work is to determine the lipophilicity parameters (R_{M0} and log P_{TLC}) of azaphenothiazines **1**–**3** by the RP TLC method, to discuss the influence of the substituents and the ring systems on the lipophilicity and to compare with the data obtained from seven computational programs.

EXPERIMENTAL

Materials

The following chemicals were used in the mobile phase: acetone (POCh, Gliwice, Poland), TRIS (tris(hydroxymethyl)aminomethane, Fluka, Switzerland)



R
a. H
b. CH_3
c. $CH_2CH{=}CH_2$
d. $CH_2C_6H_5$

Scheme 1. Phenothiazines.

and distilled water. Ethanol (POCh, Gliwice, Poland) was used for the preparation of the solutions. A set of five standards of known experimental lipophilicity ($\log P_{\text{lit.}}$) was used for a calibration curve: acetanilide (**I**) (POCh, Gliwice, Poland), 4-bromoacetophenone (**II**) (Fluka, Switzerland), benzophenone (**III**) (Fluka, Switzerland), anthracene (**IV**) (POCh, Gliwice, Poland), and p,p′-DDT (**V**) (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, obtained according to the described procedure).[11]

Selected azaphenothiazines **1**–**3** (10*H*-and 10-alkyldipyrido-1,4-thiazines **1a**–**1d**, 6*H*- and 6-alkyldiquino-1,4-thiazines **2a**–**2d** and 14*H*- and 14-alkyldiquino-1,4-thiazines **3a**–**3d** Scheme 1) were obtained in cyclizations of disubstituted pyridines and quinolines to form multicyclic thiazines followed by *N*-alkylation reactions.[12–16]

Chromatographic Procedure

Thin-layer chromatography was performed on 10 cm × 10 cm RP TLC plates precoated with silica gel RP-18F_{254S} (Merck). The mobile phase was acetone and aqueous TRIS (tris-(hydroxymethyl)aminomethane) buffer pH 7.4 (ionic strength 0.2 M). The concentration of acetone in the mobile phase ranged from 50 to 85% (v/v) in 5% increments. Azaphenothiazines **1**–**3** and standards **I**–**V** were dissolved in ethanol (2.0 mg mL^{-1}) and 2 μL of these solutions were spotted on the plates 10 mm from the bottom edges. Before development of the plates, chromatographic chambers were saturated with the mobile phase for 0.5 h. After development of the plates and drying in a stream of air, the chromatograms were observed under UV light at $\lambda = 254$ nm. At least three chromatograms were developed for each solute-solvent combination and R_F values were averaged. The R_M values calculated from experimental R_F values by use of the equation:

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

were linearly dependent on the concentration of acetone.

The R_{M0} values were obtained by extrapolation to zero acetone concentration by use of the equation:

$$R_M = R_{M0} + bC$$

where C is the concentration [%, v/v] of acetone in the mobile phase.

Computational Programs

Calculation methods are based on atomic (XLOGP),[17] atomic/fragmental (KOWWIN),[18] fragmental (CLOGP,[19] ClogP[20]), group contributions (miLogP),[21] and neural network algorithms with electrotopological-state

indices (IAlogP,[22] ALOGPS[23]) using the commercial and the internet databases.

RESULTS AND DISCUSSION

The R_M values of azaphenothiazines **1**–**3** decreased linearly with the increasing concentration of acetone in the mobile phase. Table 1 contains the R_{M0} (intercept), b (slope), and r (correlation coefficient) values for azaphenothiazines **1**–**3**. The R_{M0} values are in the range of 1.2767–5.9488 and depend strongly on the compound structure. The influence of the substituents and multicyclic ring system on the parameter R_{M0} is observed in the following order: benzyl > allyl > methyl > H, pentacene **2** > pentaphene **3** > triacene **1**.

Although one may expect from the chemical structures that pentacyclic azaphenothiazines **2** and **3** are more lipophilic than tricyclic azaphenothiazines **1**, significant differences in the R_{M0} values up to ± 2.66 in isomeric azaphenothiazines **2a**–**2d** and **3a**–**3d** are quite unexpected. These differences can be regarded as a result of their different polarity. The most lipophilic compound is **2d** ($R_{M0} = 5.9488$) and the least lipophilic is **1a** ($R_{M0} = 1.2767$), which indicates that the lipophilicity range covers five orders of magnitude.

Whereas the parameter R_{M0} describes the partitioning between non-polar stationary and polar mobile phases, the slope b describes the specific hydrophobic surface area of the tested compounds. The analysis of the equation:

$$R_{M0} = -93.900b - 0.3614 \ (r = 0.9921, s = 0.2166, \mathrm{F} = 625.1)$$

Table 1. Values of R_{M0} (intercept), b (slope), r (correlation coefficient) from the linear relationship $R_M = R_{M0} + b\mathrm{C}$ and experimental lipophilicity parameter (log P_{TLC}) for azaphenothiazines **1**–**3**

Compound	R_{M0}	$-b$	r	S	log P_{TLC}
1a	1.28	0.0201	0.9929	0.0318	1.54
1b	1.41	0.0191	0.9870	0.0369	1.69
1c	1.64	0.0227	0.9926	0.0367	1.94
1d	2.20	0.0300	0.9941	0.0434	2.57
2a	3.55	0.0418	0.9955	0.0627	4.06
2b	4.64	0.0509	0.9988	0.0393	5.28
2c	5.54	0.0608	0.9994	0.0620	6.27
2d	5.95	0.0691	0.9909	0.1489	6.73
3a	1.40	0.0157	0.9964	0.0211	1.67
3b	1.99	0.0221	0.9882	0.0541	2.33
3c	2.88	0.0358	0.9994	0.0199	3.32
3d	3.29	0.0409	0.9972	0.0479	3.78

Table 2. Comparison of literature (log $P_{lit.}$), experimental (R_{M0} and log P_{TLC}) and lipophilicity parameters for the standards used

Lipophilicity parameters	Standards				
	I	**II**	**III**	**IV**	**V**
Log$P_{lit.}$	1.21[24]	2.43[25]	3.18[25]	4.45[25]	6.38[26]
R_{M0}	1.0011	2.2592	2.6136	3.7733	5.6956
$-b$	0.0189	0.0342	0.0355	0.0490	0.0701
R	0.9971	0.9905	0.9968	0.9970	0.9913
Log P_{TLC}	1.23	2.63	3.02	4.31	6.45

Note: b (slope) and r (correlation coefficient) from the linear relationship $R_M = R_{M0} + bC$.

shows a high correlation between the R_{M0} and b values, indicating that all azaphenothiazines **1–3** can be considered as a series of compounds belonging to the same class.

In order to determine the parameter log P_{TLC} for azaphenothiazines **1–3**, a calibration curve was obtained under the same measurement conditions for a set of standards **I–V** (Table 2). Correlation between the literature values of log $P_{lit.}$ and the experimental values of R_{M0} for standards **I–V** gave the calibration equation:

$$\log P_{TLC} = 1.1113R_{M0} + 0.1161(r = 0.9971, s = 0.1747, F = 507.08, p = 0.0002)$$

The optimized chromatographic system was checked by calculations of the log P_{TLC} values for standards **I–V** using the calibration equation. The differences between the log P_{TLC} and log $P_{lit.}$ values for standards **I–V** do not exceed ± 0.2.

The obtained R_{M0} values for azaphenothiazines **1–3** were used to calculate the experimental lipophilicity parameter, log P_{TLC}, by means of the calibration equation (Table 1).

Since computational methods for calculation of log P have been recently developed, we used seven computer programs based on different theoretical approaches. Calculations of log P_{calcd} values for azaphenothiazines **1–3** gave very different results depending on the program used. Only in a few cases for azaphenothiazines **1a–1d** and **2a–2d**, the calculated values of log P_{calcd} were close to the values of log P_{TLC} obtained experimentally. The best agreement between the estimated log P_{calcd} and experimental log P_{TLC} values were obtained for azaphenothiazines **1a–1d** using the ALOGPS program; all differences were lower than ± 0.5. In the case of azaphenothiazines **3a–3d,** none of the calculation programs gave similar values of log P_{calcd} to log P_{TLC}; the differences were substantial and ranged from

Table 3. The calculated lipophilicity parameter (log P_{calcd}) for azaphenothiazines **1–3**

	log P_{calcd}						
Compound	XLOGP	KOWWIN	CLOGP	ClogP	miLogP	IAlogP	ALOGPS
1a	1.28	1.45	2.12	2.62	1.58	2.52	2.05
1b	1.59	2.00	2.21	2.62	1.93	2.58	1.86
1c	2.20	2.84	2.90	3.40	2.80	2.84	2.44
1d	3.30	3.40	4.39	4.39	3.48	3.99	3.07
2a	5.45	5.41	4.89	5.39	5.04	4.47	5.39
2b	5.18	4.49	3.65	5.39	5.16	3.50	4.19
2c	6.37	6.80	5.66	6.16	6.26	5.20	5.37
2d	7.46	7.36	7.16	7.16	6.94	7.28	6.19
3a	3.99	4.12	4.89	5.38	4.47	5.33	4.49
3b	3.72	3.21	3.65	5.39	4.59	3.43	3.21
3c	4.91	5.52	5.66	6.16	5.70	6.88	4.95
3d	6.01	6.08	7.16	7.16	6.38	7.05	5.71

± 0.84 to even ± 3.67 (Table 3). The comparison between the R_{M0} and $\log P_{calcd}$ values ($R_{M0} = b\log P_{calcd} + a$) showed good correlation coefficients but large standard errors of the estimates, which makes the correlation insignificant (Table 4).

Since the differences in $\log P_{calcd}$ values for each compound were unexpectedly significant in some cases, we checked the predictive power of these calculation programs by comparing the calculated $\log P_{calcd}$ values (Table 5) with literature values for standards **I–V**. Two programs, ALOGPS and IAlogP, estimated $\log P_{calcd}$ values with differences lower than ± 0.2 in comparison with the $\log P_{lit.}$ values. In some cases, the estimated values were the same or very similar to those taken from literature (differences $= 0 - 0.05$).

Table 4. Correlations between the R_{M0} and $\log P_{calcd}$ values for azaphenothiazines **1–3**

Program	*b*	*a*	*R*	*s*
XLOGP	0.7459	−0.2195	0.8855	0.8021
KOWWIN	0.7514	−0.3191	0.8602	0.8802
CLOGP	0.6389	0.0864	0.6629	1.2924
ClogP	0.7758	−0.8752	0.7162	1.2046
miLogP	0.7183	−0.2399	0.7993	1.0374
IAlogP	0.5237	0.5766	0.5574	1.4331
ALOGPS	0.8439	−0.4603	0.7722	1.0968

Note: *b* (slope), *a* (intercept), *r* (correlation coefficient) and *s* (standard errors) from the correlation: $R_{M0} = b \log P_{calcd} + a$.

Table 5. Comparison of calculated lipophilicity parameters log P_{calcd} for standards **I–V**

	log P_{calcd}						
Standard	XLOGP	KOWWIN	CLOGP	ClogP	miLogP	IAlogP	ALOGPS
I	1.28	1.10	1.16	1.16	1.74	1.19	1.05
II	2.66	2.56	2.52	2.52	2.74	2.46	2.43
III	3.58	3.15	3.18	3.18	3.35	3.16	3.03
IV	4.55	4.35	4.49	4.48	4.71	4.65	4.55
V	6.65	6.79	6.76	6.67	7.09	6.48	6.29

Only in two cases, the programs overestimated log P_{calcd} values with differences higher than ± 0.5, which is regarded as unacceptable.[27] The correlation between the R_{M0} and log P_{calcd} values for standards **I–V**, in contrast to azaphenothiazines **1–3**, was significant with high correlation coefficients and relatively small standard errors of the estimates (Table 6).

Although computational programs are very useful and provide valuable information for many compounds, there are some limitations to their use. The calculated log P values are not sufficiently precise in possible contributions from conformation, ionization, hydration, stereoisomerism, ion-pair formation, keto-enol tautomerism, intra- and intermolecular hydrogen-bond formation, folding, etc. When the calculation fails badly, there are strong indications that it is conformational information which is lacking. The information may be even more valuable than the lipophilic parameter itself.[1] The predictive power of the computational programs was quite good for the standards **I–V**, but rather weak for azaphenothiazines **1–3** (Figure 1). As was determined by X-ray analysis of selected azaphenothiazines **1–3**, the multicyclic ring systems are not planar; the central thiazine ring is in a boat

Table 6. Correlations between the R_{M0} and log P_{calcd} values for standards **I–V**

Program	*b*	*a*	*R*	*s*
XLOGP	0.8705	−0.1907	0.9948	0.2090
KOWWIN	0.8175	0.1483	0.9993	0.0772
CLOGP	0.8322	0.0544	0.9920	0.0818
ClogP	0.8452	0.02251	0.9990	0.0894
miLogP	0.8520	−0.2765	0.9964	0.1730
IAlogP	1.8631	−0.0282	0.9962	0.1792
ALOGPS	0.8756	0.0215	0.9957	0.1900

Note: *b* (slope), *a* (intercept), *r* (correlation coefficient) and *s* (standard errors) from the correlation: $R_{M0} = b \log P_{calcd} + a$.

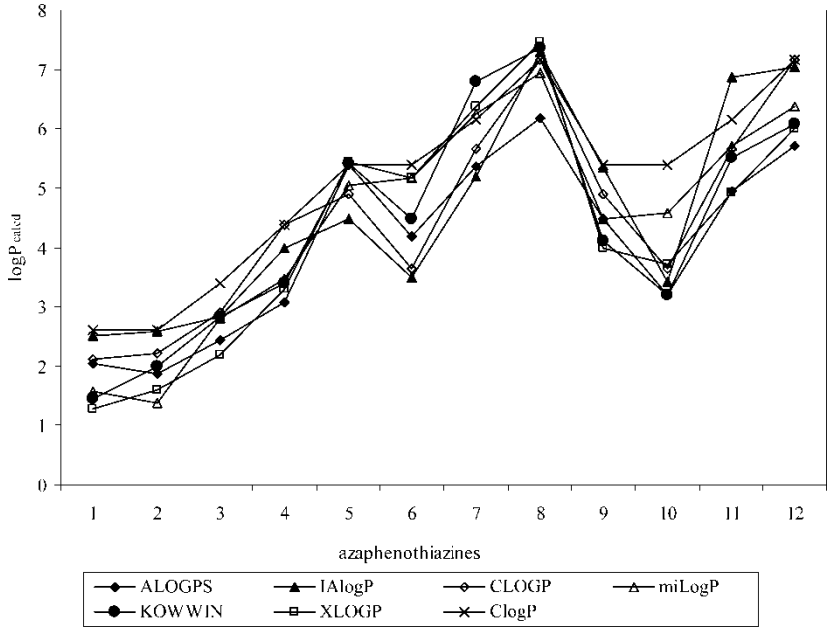


Figure 1. Score plot of the $\log P_{\text{calcd}}$ values for azaphenothiazines **1**–**3** obtained using various calculating programs.

conformation with the substituent in quasi-equatorial position[14,28] or in quasi-axial position.[29] It seems that the folding conformation of azaphenothiazines **1**–**3** makes it difficult to obtain reliable values of $\log P_{\text{calcd}}$.

CONCLUSION

RP TLC is a powerful method for determination of the lipophilicity parameters R_{M0} and $\log P_{TLC}$, even for extremely lipophilic compounds (for example for compound **2d**: $R_{M0} = 5.9488$, $\log P_{TLC} = 6.73$ and for compound **V**: $R_{M0} = 5.6956$, $\log P_{TLC} = 6.45$). The parameter R_{M0} and specific hydrophobic surface area b were significantly intercorrelated showing a congeneric class of azaphenothiazines **1**–**3**. The R_{M0} values were converted into the $\log P_{TLC}$ values by use of the calibration curve obtained for the standards. Linear condensed azaphenothiazines **2** were much more lipophilic than angular condensed isomers **3**. Although the experimental determination of $\log P$ can be replaced by the calculation of $\log P_{\text{calcd}}$ for relatively simple compounds (for examples standards **I**–**V**) using computational programs, for more complicated compounds the calculation demands to check its validity by comparison with experimental data, for example from the RP TLC method.

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