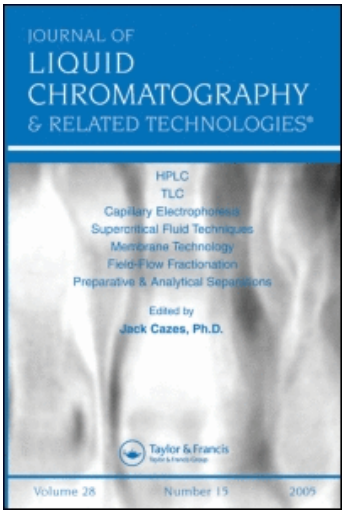

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RP TLC Determination of the Lipophilicity of New 10-Substituted 2,7-Diazaphenothiazines*

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Abstract: Experimental (R_{M0} and log P_{TLC}) and calculated (log P_{calcd}) lipophilicity parameters of eleven new bioactive 10-substituted 2,7-diazaphenothiazines **2**–**12** 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, and obtained by the calculation program miLogP. The parameter R_{M0} was converted into parameter log P_{TLC} by use of a calibration curve obtained for five standards. The lipophilicity parameters were intercorrelated and discussed in the terms of structure-lipophilicity relationships. The parameter R_{M0} and specific hydrophobic surface area *b* were significantly intercorrelated showing three congeneric subclasses of 2,7-diazaphenothiazines **2**–**12**. The 2,7-diazaphenothiazine system turned out to be less lipophilic than the classical phenothiazine one.

Keywords: 2,7-Diazaphenothiazines, Phenothiazines, Reversed-phase TLC, Lipophilicity, R_{MO}, Log *P*

INTRODUCTION

Phenotiazines are widely known for their chemical properties and very significant biological activities (antipsychotic, antihistaminic, antiasthmatic, antiemetic, and antitussive).[1] Recent reports have focused interests on anticancer, antiplasmid, and antimicrobial activities, reversal of multidrug

*Part CIII in the series of Azinyl Sulfides.

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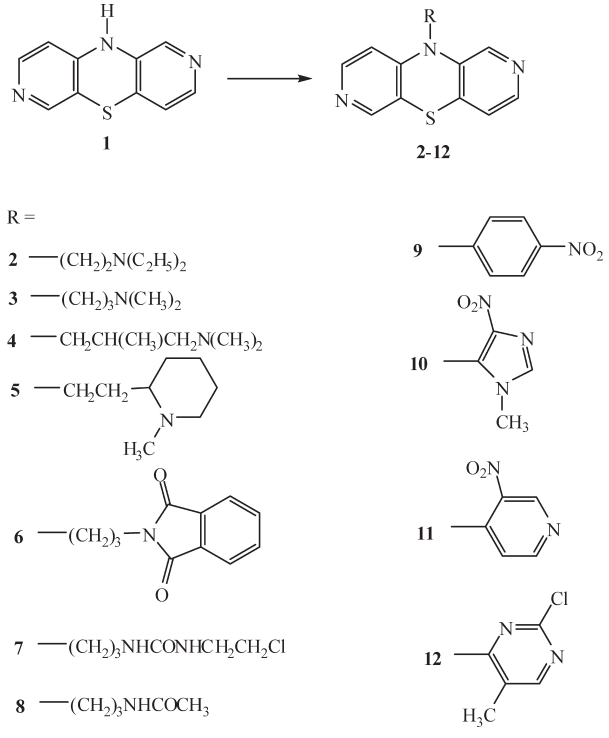
resistance (MDR), and potential treatment in Alzheimer's and Creutzfeldt-Jakob diseases.[2–5] Some modifications of the phenothiazine structures were made by substitution of the benzene ring with an azine ring. In continuation of our search for pharmacoactive pyridine and quinoline derivatives, we modified the phenothiazine structure with the pyridine ring to form a new type of the dipyrido-1,4-thiazine derivatives. The obtained 10-substituted 2,7-diazaphenothiazines **2–12** (Scheme 1)[6–8] exhibit potential antipsychotic, anticancer, antiviral, antiprotozoal, antileishmanial, antiinflammatory, and spasmolytic activity.[9] The basic compound, 10*H*-2,7-diazaphenothiazine **1**, was tested against 57 human cancer lines in the National Cancer Institute in Bethesda, showing promising activity against lung cancers HOP-62 and HOP-92, colon cancers COLO-205 and HCT-116, renal cancers RXF-393 and A-498, and leukemia HL-60(TB).[10]

Lipophilicity is a key property used in QSAR studies and in the design of new drugs with the required biological activity. Lipophilicity is generally defined as the tendency of a chemical compound to distribute between an immiscible non-polar organic solvent and water. Determination of the lipophilicity parameter, expressed as log *P*, is based on the partition of a compound in



Scheme 1. 10-Substituted 2,7-diazaphenothiazines.

the reference n-octanol-water system. The classical method of determination of log *P*, 'shake flask', is troublesome, time consuming, and limited to extremely pure compounds.[11–13] Therefore, this method has recently been replaced by other experimental methods, most often by reversed-phase chromatographic methods (RP TLC and RP HPLC),[14–17] because they are simple, rapid, easy to perform, relatively inexpensive, and do not need large amounts of very pure compounds. The obtained R_M values in the RP TLC method (from the R_F values) are extrapolated to zero concentration of organic modifier to give the R_{M0} values, which are widely used as a chromatographic alternative parameter to the log *P* values (describing partitioning between non-polar stationary and polar mobile phases)[14] or are calculated to the log P_{TLC} values using a calibration curve with standards of known lipophilicity.[15,17]

As one of the characteristic features of phenothiazines is their high lipophilic nature with log *P*, up to 5.9,[18,19] it is important to determine the lipophilicity of new phenothiazine derivatives. The purpose of this work is to determine the lipophilicity parameters (R_{M0}, log P_{TLC}, and log P_{calcd}) of 10-substituted 2,7-diazaphenothiazines **2**–**12** by RP TLC, and a computational method to discuss the influence of the substituents and the diazaphenothiazine system on the lipophilicity, and to compare the experimental and calculated data.

EXPERIMENTAL

Materials

Acetone (POCh, Gliwice, Poland), TRIS (tris(hydroxymethyl)aminomethane (Fluka, Switzerland), and distilled water were used in the mobile phase. Ethanol (POCh, Gliwice, Poland) was used for the preparation of the solutions. A set of five standards of known experimental lipophilicity (log *P*) 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.[20]

Chemistry

10-Substituted 2,7-diazaphenothiazines **2**–**12** were obtained from 10*H*-2,7-diazaphenothiazine **1** in the reactions of *N*-arylation and *N*-alkylation with appropriate alkyl and aryl halides, according to previously described procedures.[6,7] The phthalimidopropyl derivative **6** was transformed into the chloroethylureidopropyl and acetylaminopropyl derivatives **7** and **8** in the reaction of hydrolysis, followed by reaction with 2-chloroethyl isocyanate or with acetyl chloride.[8]

Thin-Layer Chromatography

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. Standards **I**–**V** and 2,7-diazaphenothiazines **2**–**12** were dissolved in ethanol (2.0 mg mL^{-1}) and 1 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 and 365 nm. At least three chromatograms were developed for each solute solvent combination and R_F values were averaged. The R_M values were 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 Program

The calculation program, miLogP, based on the sum of fragmental contributions and correction factors was used to estimate log P_{calcd}.[21]

RESULTS AND DISCUSSION

The R_M values of 2,7-diazaphenothiazines **2**–**12** 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. The correlation coefficients ranged from 0.9852 (compound **10**) to 0.9992 (compound **5**). The R_{M0} values depend strongly on the nature of the substituent at position 10 and are in the range of 1.10–2.39.

The R_{M0} values were calculated into the log P_{TLC} values by use of the calibration equation based on the standards **I**–**V**, which were measured in the same chromatographic conditions as 2,7-diazaphenothiazines **2**–**12** and described in our previous paper.[22] Correlation of the literature log P and

Table 1. Values of experimental R_{M0} (intercept), b (slope), r (correlation coefficient) from the linear relationship $R_M = R_{M0} + bC$, experimental log P_{TLC} and calculated log P_{calcd} for 10-substituted 2,7-diazaphenothiazines **2**–**12**

Compound	R_{M0}	$-b$	r	s	log P_{TLC}	log P_{calcd}
2	1.54	0.0208	0.9960	0.0241	1.83	2.46
3	1.61	0.0220	0.9915	0.0381	1.91	1.94
4	1.67	0.0245	0.9930	0.0369	1.97	2.32
5	1.59	0.0217	0.9940	0.0327	1.87	2.91
6	2.39	0.0218	0.9960	0.0258	2.78	3.37
7	1.31	0.0191	0.9882	0.0390	1.57	2.61
8	1.41	0.0204	0.9893	0.0397	1.68	1.30
9	2.10	0.0243	0.9913	0.0597	2.45	2.98
10	1.10	0.0161	0.9852	0.0370	1.34	1.73
11	1.52	0.0235	0.9954	0.0329	1.81	1.87
12	2.27	0.0201	0.9924	0.0426	2.64	3.21

R_{M0} values for a set of standards gave the calibration equation:

$$\log P_{TLC} = 1.1113R_{M0} + 0.1161\ (r = 0.9971, s = 0.1747, F = 507.08, p = 0.002)$$

Differences between the log P_{TLC} and the literature log P values for standards **I**–**V** were small and did not exceed ± 0.2 (Table 2).

The calculated log P_{TLC} values by use of the calibration equation are in the range of 1.34–2.78. As expected, the most lipophilic 2,7-diazaphenothiazines is the phthalimidopropyl derivative **6** (log $P_{TLC} = 2.78$), and a little less lipophilic are chloromethylpyrimidinyl **12** (log $P_{TLC} = 2.64$) and p-nitrophenyl **9** (log $P_{TLC} = 2.45$ derivatives. The least lipophilic are the chloroethylureidopropyl derivative **7** (log$P_{TLC} = 1.34$) and the acetylaminopropyl derivative **8** (log $P_{TLC} = 1.68$). It is worth noting that the parent compound, 10*H*-2,7-diazaphenothiazine **1**, showed log $P_{TLC} = 1.54$.[22] The 2,7-diazaphenothiazine system is less lipophilic than the classical phenothiazine one. The diaza-analog of promazine, 10-(3′-dimethylaminopropyl)-2,7-diazaphenothiazine **4** shows log $P_{TLC} = 1.97$, when promazine and chlorpromazine 2.93 and 3.26 at pH = 7.4, respectively.[19] It appears that incorporation of two nitrogen atoms into phenothiazine moiety reduces the lipophilic character.

Whereas, the parameter R_{M0} describes the partitioning between non-polar and polar phases, the slope b describes specific hydrophobic surface area of the tested compounds. Analysis of the relationship between these two parameters:

$$R_{M0} = Bb + a$$

Table 2. Values of literature log P, experimental R_{M0} (intercept), b (slope), r (correlation coefficient) from the linear relationship $R_M = R_{M0} + bC$ and experimental log P_{TLC} for standards **I**–**V**

Standards	log P	$R_{M0}^{[22]}$	log $P_{TLC}^{[22]}$	log $P_{calcd}^{[22]}$
I	1.21[23]	1.00	1.23	1.74
II	2.43[24]	2.26	2.63	2.74
III	3.18[24]	2.61	3.02	3.35
IV	4.45[24]	3.77	4.31	4.71
V	6.38[25]	5.70	6.45	7.09

is the basic feature of the chromatographic determination of lipophilicity,[14] and revealed for all 2,7-diazaphenothiazines 2–12 ($r = 0.4639$) the presence of the expected congeneric subclasses: dialkylaminoalkyl **2**–**5** ($r = 0.9669$), acylaminoalkyl (amidoalkyl) **6**–**8** ($r = 0.9138$) and aryl **9**–**12** ($r = 0.5439$) derivatives (Table 3). The low correlation coefficient of the aryl derivatives is probably due to their various systems (homoaromatic and heteroaromatic).

Since the computational methods based on various approaches have been extensively developed, we calculated the theoretical lipophilicity parameters, log P_{calcd}, for standards **I**–**V** and 2,7-diazaphenothiazines **2**–**12**, by a use of the miLogP program. Correlation between parameters log $P_{lit.}$, log P_{TLC}, and log P_{calcd} for the standards **I**–**V** gave equations with high correlation coefficients:

$$\log P_{lit.} = 0.9973 \log P_{calcd} - 0.1963 \, (r = 0.9948)$$

$$\log P_{TLC} = 0.9442 \log P_{calcd} - 0.1809 \, (r = 0.9953)$$

The highest difference between the log P_{TLC} and log P_{calcd} values is 0.64 for extremely lipophilic standard **V** (log $P_{TLC} = 6.45$) and does not exceed 10% of the value.

In contrast to the standards, the correlation between the log P_{TLC} and log P_{calcd} values for diazaphenothiazines **2**–**12** was less significant.

$$\log P_{TLC} = 0.5266 \log P_{calcd} + 0.7083 \, (r = 0.7739)$$

Table 3. Relationship between R_{M0} (intercept) and b (slopes) for 10-substituted 2,7-diazaphenothiazines **2**–**12**

Compounds	a	$-B$	r
2–**12**	0.0478	76.788	0.4639
2–**5**	0.8899	32.035	0.9669
6–**8**	−6.5348	403.090	0.9138
9–**12**	0.1095	78.105	0.5439

The predictive power of the computational program was quite good for simple standards **I**–**V**, but rather moderate for diazaphenothiazines **2**–**12**. According to these calculations, the most lipophilic 2,7-diazaphenothiazine was, once more, the phthalimidopropyl derivative **6** (log $P_{calcd} = 3.37$) and the least lipophilic was the acetylaminopropyl derivative **8** (log $P_{calcd} = 1.30$). The highest differences between the log P_{TLC} and log P_{calcd} values is 1.04 for diazaphenothiazines **5** and **7**. The calculated log P_{calcd} values are not sufficiently precise in more complicated compounds where possible contributions are mainly from conformation and molecule folding.[12] The 2,7-diazaphenothiazine system is folding with the central thiazine ring in a boat conformation and the substituent in the quasi-equatorial position, as was revealed in X-ray analysis of compound **11**.[6]

CONCLUSION

The lipophilicity parameters R_{M0}, log P_{TLC}, and log P_{calcd} were determined for a new type of phenothiazines, 10-substituted 2,7-diazaphenothiazines **2**–**12**. The log P_{TLC} values for the derivatives **2**–**5** with pharmacophoric dialkylaminoalkyl substituents are lower than the values for appropriate neuroleptic phenothiazine drugs, which may suggest other than neuroleptic biological activities. The incorporation of two nitrogen atoms in positions 2 and 7 into phenothiazine moiety reduces the lipophilic character. Significant correlation between R_{M0} and specific hydrophobic surface area *b* for three congeneric subclasses of 2,7-diazaphenothiazines **2**–**12** was found.

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