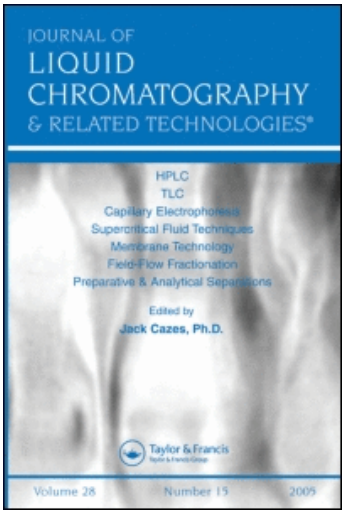

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LIPOPHILICITY CHARACTERIZATION BY REVERSED-PHASE HPLC OF POTENTIAL ANTITUBERCULOTICS

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ABSTRACT

Lipophilicity is one of the properties which influence the partition of a substance in biological media. The reversed-phase high-performance liquid chromatographic (RP-HPLC) capacity factors k of two series of 2-benzylthioderivatives, newly synthesized as potential antituberculous drugs, were determined on a C-18 stationary phase with methanol–water as the mobile phase, using UV detection. The measured $\log k$ values were compared with the $\log P$ values obtained by means of a mathematical method. High correlation was found between $\log P$ and $\log k$ values.

INTRODUCTION

Lipophilicity, a medicinally relevant physico-chemical property, plays a basic role in many biological processes. Lipophilicity is generally defined and

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usually measured by the partition coefficient of the organic compound between an immiscible non-polar solvent and water.[1] Fujita et al. have proposed the *n*-octanol–water partition coefficient, $P_{o/w}$, as a measure of a compound's lipophilicity.[2] The logarithm of the partition coefficient of a chemical in the *n*-octanol/water system ($\log P$), which is usually measured by "shake-flask" method, is widely used because of its simplicity and some similarity between *n*-octanol and biological membranes.[3] However, practical disadvantages and the limitation to $\log P$ values between -2 and $+4$, led the researchers to investigate other methods for lipophilicity measurements.[4,5]

These shortcomings are overcome by chromatographic methods, which are very important alternatives to *n*-octanol/water partitioning. They are rapid and relatively simple, very small quantities of substances are required, and the compounds need not be very pure. Lipophilicity can be determined by reversed-phase high-performance liquid chromatography (RP-HPLC)[3,6–11] and by reversed-phase thin-layer chromatography.[11–17] Recent research indicates that both methods are equally suitable for this purpose.

It has been demonstrated that the retention capacity factor k of a compound in RP-HPLC system is a reliable indirect descriptor of lipophilicity of a compound.[3] The retention capacity factor is given by $k = (t_r - t_0)/t_0$, where t_r and t_0 are the retention times of the solute and the unretained compound, respectively. Moreover, some studies have shown that $\log k_w$, the retention factor, which is extrapolated from the binary phase to 100% water in an RP-HPLC system, is an even better descriptor of lipophilicity than an isocratic factor, because it is independent of any organic modifier effects, and it reflects polar–non-polar partitioning in a manner similar to the "shake-flask" measurement.[11,18–20]

The present paper aims at the RP-HPLC evaluation of lipophilicity of a series of newly prepared potential antituberculous drugs and a comparison of experimentally measured values with the theoretically calculated $\log P$ values, by means of a computer program.

EXPERIMENTAL

Instruments

The HPLC system consisted of a LCP 4100 pump (Ecom, Prague, Czech Republic), a C6W VICI AG manual injector (Valco Europe, Switzerland), and a UV detector LCD 2084 (Ecom, Prague, Czech Republic). A chromatography station for Windows Version CSW 1.7 DLL (Data Apex, Czech Republic) was used for peak registration and calculation of retention time. The stationary phase was LiChrosphere 100 RP-18, 125×4 mm I.D., 5 μm (Merck, Germany).

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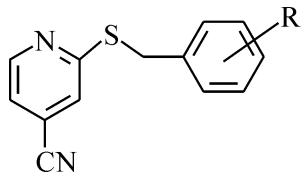
Chemicals

The structures of the thirty 2-benzylthioderivatives under examination are shown in Tables 1 and 2. These compounds were previously synthesized as potential antituberculous drugs.[21] Stock solutions of all compounds were made up in HPLC grade methanol to a concentration of approximately 0.1 mg/mL.

Measurement of Log k

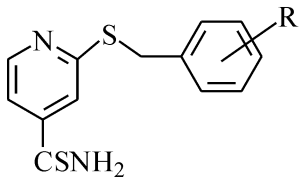
The mobile phases were made by mixing methanol with water in proportions 50 : 50, 55 : 45, 60 : 40, 70 : 30 (v/v). The optimal composition of the mobile phase for both series was methanol–water, (45 : 55)(v/v). The flow

Table 1. Structure of 2-Benzylthioderivatives of Pyridine-4-Carbonitrile



Compound	R	Empirical Formula	M_r
A-01	H	$C_{13}H_{10}N_2S$	226.3
A-02	2-Cl	$C_{13}H_9ClN_2S$	260.7
A-03	3-Cl	$C_{13}H_9ClN_2S$	260.7
A-04	4-Cl	$C_{13}H_9ClN_2S$	260.7
A-05	2-F	$C_{13}H_9FN_2S$	244.3
A-06	3-F	$C_{13}H_9FN_2S$	244.3
A-07	4-F	$C_{13}H_9FN_2S$	244.3
A-08	3-Br	$C_{13}H_9BrN_2S$	305.2
A-09	4-Br	$C_{13}H_9BrN_2S$	305.2
A-10	3-CH_3	$C_{14}H_{12}N_2S$	240.3
A-11	4-CH_3	$C_{14}H_{12}N_2S$	240.3
A-12	4-NO_2	$C_{13}H_9N_3O_2S$	271.3
A-13	2,4-NO_2	$C_{13}H_8N_4O_4S$	316.3
A-14	2,6-F,Cl	$C_{13}H_8ClFN_2S$	278.7
A-15	2,6-F,NO_2	$C_{13}H_8FN_3O_2S$	289.3
A-16	4-OCH_3	$C_{14}H_{12}N_2OS$	256.3

Table 2. Structure of 2-Benzylthioderivatives of Pyridine-4-Carbothioamide



Compound	R	Empirical Formula	M_r
B-01	H	$C_{13}H_{12}N_2S_2$	260.4
B-02	2-Cl	$C_{13}H_{11}ClN_2S_2$	294.8
B-03	4-Cl	$C_{13}H_{11}ClN_2S_2$	294.8
B-04	2-F	$C_{13}H_{11}FN_2S_2$	278.4
B-05	3-F	$C_{13}H_{11}FN_2S_2$	278.4
B-06	4-F	$C_{13}H_{11}FN_2S_2$	278.4
B-07	3-Br	$C_{13}H_{11}BrN_2S_2$	338.0
B-08	4-Br	$C_{13}H_{11}BrN_2S_2$	338.0
B-09	3-CH_3	$C_{14}H_{14}N_2S_2$	274.4
B-10	4-CH_3	$C_{14}H_{14}N_2S_2$	274.4
B-11	4-NO_2	$C_{13}H_{11}N_3O_2S_2$	305.4
B-12	2,6-F,Cl	$C_{13}H_{10}ClFN_2S_2$	312.8
B-13	2,6-F,NO_2	$C_{13}H_{10}FN3O_2S_2$	323.4
B-14	4-OCH_3	$C_{14}H_{14}N_2OS_2$	290.4

rate was 1 mL/min. A methanolic solution of potassium iodide was used for t_0 measurement.

RESULTS AND DISCUSSION

Reversed-phase high-performance liquid chromatographic conditions were found, making possible isocratic elution of all tested drugs in an acceptable period of time, and with sufficient mutual differences t_r. Values k and $\log k$ were determined for all compounds through the RP-HPLC measurements, as described in the experimental section. Experimentally measured $\log k$ values were compared with theoretically calculated $\log P$ values, which were obtained on the software ACD/Log *P*, Version 1.0, Toronto (1994, 1995). Correlation and regression analysis of $\log P$ and $\log k$ were run on a PC computer using the Microsoft Excel program.

Table 3. Log P, t_r, k and log k Values of 2-Benzylthioderivatives of Pyridine-4-Carbonitrile

Compound	R	t_r	k	log k	log P
A-01	H	69.24	85.55	1.93	3.28
A-02	2-Cl	172.57	214.71	2.33	3.87
A-03	3-Cl	179.93	223.91	2.35	3.87
A-04	4-Cl	184.47	229.58	2.36	3.87
A-05	2-F	84.04	104.05	2.02	3.33
A-06	3-F	78.56	97.20	1.99	3.33
A-07	4-F	81.14	100.43	2.00	3.33
A-08	3-Br	204.51	254.64	2.41	4.05
A-09	4-Br	219.85	273.81	2.44	4.05
A-10	3-CH_3	150.19	186.74	2.27	3.74
A-11	4-CH_3	159.94	198.93	2.30	3.74
A-12	**4-NO_2**	**56.22**	**69.28**	**1.84**	**3.01**
A-13	**2,4-NO_2**	**54.78**	**67.48**	**1.83**	**2.68**
A-14	2,6-F,Cl	172.26	214.33	2.33	3.48
A-15	2,6-F, NO_2	64.79	79.99	1.90	2.96
A-16	4-OCH_3	71.67	88.59	1.95	3.19

Tables 3 and 4 sum up all results obtained from both RP-HPLC measurements and, by the calculation by the means of the above-mentioned program and methods, for both series of 2-benzylthioderivatives. The values of k and log k are listed here for the optimal chromatographic conditions for each series tested. For the series of 2-benzylthioderivatives of pyridine-4-carbonitrile, the values k and log k measured on the stationary phase, LiChrosphere, ranged between 67.48–273.81 and 1.83–2.44, respectively. The lowest values of the capacity factor were found by compound A-13 (2,4-NO_2). On the other hand, the highest retention was shown by compound A-09 (4-Br). For the series of 2-benzylthioderivatives of pyridine-4-carbothioamide, the values k and log k were measured on the stationary phase, LiChrosphere, ranging between 43.39–162.66 and 1.64–2.21, respectively. The lowest values of the capacity factor were found in compound B-11 (4-NO_2); on the other hand, the highest retention was shown by compound B-08 (4-Br).

The calculated values of log P were compared with the measured values of log k. Good correlation was observed for between log P and log k values. For both series of 2-benzylthioderivatives, the dependences of log P were demonstrated with a reliability of 99.9%. Equation of correlation dependence for the series A: $\log P = 1.75\ \log k - 0.26$; $n = 16$; $r = 0.948$; $s = 0.137$; $F = 61.6$. Equation of correlation dependence for the series B: $\log P = 1.69\ \log k$; $n = 14$; $r = 0.951$; $s = 0.118$; $F = 56.9$.

Table 4. Log P, t_r, k and log k Values of 2-B Benzylthioderivatives of Pyridine-4-Carbothioamide

Compound	R	t_r	k	log k	log P
B-01	H	39.18	47.98	1.68	2.98
B-02	2-Cl	93.88	116.35	2.07	3.58
B-03	4-Cl	107.00	132.75	2.12	3.58
B-04	2-F	47.27	58.09	1.76	3.03
B-05	3-F	48.28	59.35	1.77	3.03
B-06	4-F	48.00	59.00	1.77	3.03
B-07	3-Br	118.49	147.11	2.17	3.75
B-08	4-Br	130.93	162.66	2.21	3.75
B-09	3-CH_3	72.86	90.08	1.95	3.44
B-10	4-CH_3	83.96	103.95	2.02	3.44
B-11	4-NO_2	35.51	43.39	1.64	2.71
B-12	**2,6-F,Cl**	**90.95**	**112.69**	**2.05**	**3.19**
B-13	2,6-F,NO_2	37.96	46.45	1.67	2.66
B-14	4-OCH_3	39.51	48.39	1.68	2.90

Table 5. Deviating Compounds of the Series of 2-Benzylthioderivatives of Pyridine-4-Carbonitrile

Deviating Compound	R	Log P	Log k	Included in Correlation	Excluded from Correlation
A-13	2,4-NO_2	2.68	1.83	0.948	0.952
A-14	2,6-F,Cl	3.48	2.33	0.948	0.974
A-13 and A-14	2,4-NO_2 and 2,6-F,Cl	2.68 and 3.48	1.83 and 2.33	0.948	0.986

Table 6. Deviating Compound of the Series of 2-Benzylthioderivatives of Pyridine-4-Carbothioamide

Deviating Compound	R	Log P	Log k	Included in Correlation	Excluded from Correlation
B-12	2,6-F,Cl	3.19	2.05	0.951	0.979

In the correlation of $\log P$ and $\log k$ values in both series, some compounds were found, which deviated from correlation dependences. These deviating compounds are summed up in Tables 5 and 6. Besides $\log P$ and $\log k$ values, correlation coefficients of regression dependences are listed here for the case when the compound was included in correlation and for the case when it was excluded from correlation. "Deviating" compounds are also marked out in Tables 3 and 4 (boldface). In the series of carbonitrile derivatives, compounds A-13 (2,4-NO_2) and A-14 (2,6-F, Cl), markedly deviated in correlations. In the series of carbothioamide derivatives, compound B-12 (also 2,6-F, Cl) markedly deviated.

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