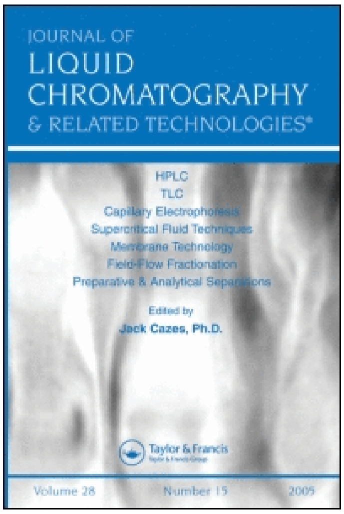

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DETERMINATION OF LIPOPHILICITY OF POTENTIAL ANTITUBERCULOUS DRUGS BY REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The RP-HPLC capacity factors k of two series of 4-benzylthio-derivatives, newly synthetised as potential antituberculous drugs, were determined on two types of C18 columns 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 mathematical programmes and methods. High correlation was found between log P and log k values.

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INTRODUCTION

Lipophilicity, a medicinally relevant physico-chemical property, plays a basic role in many biological processes. Lipophilicity is generally defined as the tendency of a chemical to distribute between an immiscible non-polar solvent and water. The logarithm of the partition coefficient of a chemical in the n-octanol/water system (K_{ow}), which is usually measured by “shake flask” method, is widely used because of its simplicity and some similarity between n-octanol and biological membranes.(1) However, practical disadvantages and the limitation to log K_{ow} values between –2 and +4 led the researchers to investigate other methods for lipophilicity measurements(2,3).

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)(1,4-7) and by reversed-phase thin-layer chromatography(8-12). 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 the lipophilicity of a compound.(1) 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 RP-HPLC system, is an even better descriptor of lipophilicity than the isocratic factor because it is independent of any organic modifier effects, and it reflects polar–non-polar partitioning in a manner similar to “shake flask” measurement(13-15).

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 computer programmes and methods.

EXPERIMENTAL

Instruments

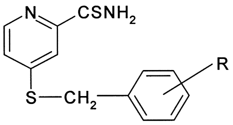
The HPLC system consisted of a SP8700 pump, a SP8750 sampler (both Spectra Physics, USA), and a UV detector set at 254 nm (Ecom, Czech Republic). Chromatography Station for Windows Version CSW 1.7 DLL (Data Apex, Czech Republic) was used for peak registration and calculation of retention time. The stationary phases were Separon SGX C18, 150 x 3 mm I.D., 7 µm

(Tessek, Czech Republic) and LiChrosphere 100 RP-18, 125 x 4 mm I.D., 5 µm (Merck, Germany).

Chemicals

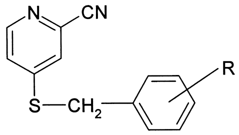
The structures of the examined 36 4-benzylthioderivatives are shown in Tables 1 and 2. These compounds were previously synthetized as potential antituberculous drugs.(16) Stock solutions of all compounds were made up in HPLC grade methanol to a concentration of approximately 1 mg/mL.

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



Compound	R	Empirical Formula	M_r
T1	H	$C_{13}H_{11}N_2S_2$	260.4
T2	2-Cl	$C_{13}H_{11}ClN_2S_2$	294.8
T3	3-Cl	$C_{13}H_{11}ClN_2S_2$	294.8
T4	4-Cl	$C_{13}H_{11}ClN_2S_2$	294.8
T5	2-F	$C_{13}H_{11}FN_2S_2$	278.4
T6	3-F	$C_{13}H_{11}FN_2S_2$	278.4
T7	4-F	$C_{13}H_{11}FN_2S_2$	278.4
T8	3-Br	$C_{13}H_{11}BrN_2S_2$	338.0
T9	3-CH_3	$C_{14}H_{14}N_2S_2$	274.4
T10	4-CH_3	$C_{14}H_{14}N_2S_2$	274.4
T11	3-NO_2	$C_{13}H_{11}N_3O_2S_2$	305.4
T12	4-NO_2	$C_{13}H_{11}N_3O_2S_2$	305.4
T13	2-F,6-NO_2	$C_{13}H_{10}FN_3O_2S_2$	323.4
T14	2-F,6-Cl	$C_{13}H_{10}ClF\ N_2S_2$	312.8
T15	4-OCH_3	$C_{14}H_{14}N_2OS_2$	290.4
T16	3,4-Cl	$C_{13}H_{10}Cl_2\ N_2S_2$	328.0
T17	3,4-F	$C_{13}H_{10}F_2\ N_2S_2$	296.0
T18	2,4-NO_2	$C_{13}H_{10}N_4O_4S_2$	350.4
T19	3,5-NO_2	$C_{13}H_{10}N_4O_4S_2$	350.4

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



Compound	R	Empirical Formula	M_r
N1	H	$C_{13}H_{10}N_2S$	226.3
N2	2-Cl	$C_{13}H_9ClN_2S$	260.7
N3	3-Cl	$C_{13}H_9ClN_2S$	260.7
N4	4-Cl	$C_{13}H_9ClN_2S$	260.7
N5	2-F	$C_{13}H_9FN_2S$	244.3
N6	3-F	$C_{13}H_9FN_2S$	244.3
N7	3-Br	$C_{13}H_9BrN_2S$	305.2
N8	4-Br	$C_{13}H_9BrN_2S$	305.2
N9	3-CH_3	$C_{14}H_{12}N_2S$	240.3
N10	3-NO_2	$C_{13}H_9N_3O_2S$	271.3
N11	4-NO_2	$C_{13}H_9N3O_2S$	271.3
N12	2-F,6-NO_2	$C_{13}H_8FN_3O_2S$	289.3
N13	2-F,6-Cl	$C_{13}H_8ClF\ N_2S$	278.7
N14	4-OCH_3	$C_{14}H_{12}N_2OS$	256.3
N15	3,4-Cl	$C_{13}H_8Cl_2\ N_2S$	295.2
N16	3,4-F	$C_{13}H_8F_2\ N_2S$	262.3
N17	2,4-NO_2	$C_{13}H_8N_4O_4S$	316.3

Measurement of Log k

The mobile phases were made by mixing methanol with water in the proportions 50:50, 55:45, 60:40, 70:30 (v/v). The optimal composition of the mobile phase for the series of 4-benzylthioderivatives of pyridine-2-carbothioamides was methanol–water 55:45 (v/v) and for a series of 4-benzyltioderivatives of pyridine-2-carbonitriles methanol–water, 60:40 (v/v). The flow rate was 1 mL/min. A methanolic solution of potassium iodide was used for the measurement of t_0.

RESULTS AND DISCUSSION

RP-HPLC chromatographic conditions were found, which make 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 have been 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 HyperChem Suite for Windows (release 5.1), the module ChemPlus 1.6 using atomic parameters derived by Ghose, Pritchett and Crippen,(17) and later extended by Viswanadhan a coworkers.(18) The program Chem3D, which was used for further calculations of log P, uses three different methods. The first one was published by Ghose and Crippen,(19) the second method by Viswanadhan and coworkers,(18) and the third one by Broto, Moreau and Vandycke.(20) Correlation and regression analyses of log P and log k were run on a PC computer using the Microsoft Excel program.

Tables 3 and 4 sum up all results obtained from both RP-HPLC measurements and by the calculation by means of the above-mentioned programmes and methods for both series of 4-benzylthioderivatives. The values of k and log k are listed here for the optimal chromatographic conditions for each series tested. For

Table 3. Log P and Log k Values for the Series of 4-Benzylderivatives of Pyridine-2-carbothioamide

		Log P				Stationary Phase			
		Mathematical Method				Separon		LiChrospher	
Compound	R	Crippen[19]	Viswan.[18]	Broto[20]	HyperCh.[17]	k	Log k	k	Log k
T1	H	2.76	**2.74**	2.00	**2.65**	111.74	**2.05**	12.39	1.09
T2	2-Cl	3.32	3.26	2.62	3.17	100.43	2.00	22.16	1.35
T3	3-Cl	3.32	3.26	2.62	3.17	92.80	1.97	21.14	1.33
T4	4-Cl	3.32	3.26	2.62	3.17	66.61	1.82	23.26	1.37
T5	2-F	2.92	2.88	2.14	2.79	53.94	1.73	12.79	1.11
T6	3-F	2.92	2.88	2.14	2.79	50.75	1.71	12.37	1.09
T7	4-F	2.92	2.88	2.14	2.79	27.95	1.45	12.82	1.11
T8	3-Br	3.59	3.53	2.89	3.45	106.39	2.03	24.41	1.39
T9	3-CH_3	3.25	3.21	2.42	3.12	100.12	2.00	23.39	1.37
T10	4-CH_3	3.25	3.21	2.42	3.12	70.04	1.85	26.04	1.42
T11	3-NO_2	2.05	2.56	0.65	2.61	27.84	1.44	6.87	0.84
T12	4-NO_2	2.05	2.56	0.65	2.61	20.22	1.31	7.58	0.88
T13	2-F,6-NO_2	2.21	2.70	0.78	2.75	20.82	1.32	7.42	0.87
T14	2-F,6-Cl	3.48	3.40	2.76	3.31	103.11	2.01	22.19	1.35
T15	4-OCH_3	2.63	**2.49**	2.13	**2.40**	38.05	**1.58**	13.35	**1.13**
T16	3,4-Cl	3.88	3.78	3.24	3.69	174.44	2.24	38.77	1.59
T17	3,4-F	3.08	3.02	2.27	2.93	38.72	1.59	13.75	1.14
T18	2,4-NO_2	1.34	2.39	−0.71	2.56	17.84	1.25	7.17	0.86
T19	3,5-NO_2	1.34	2.39	−0.71	2.56	22.83	1.36	6.40	0.81

Table 4. Log P and Log k Values for the Series of 4-Benzylthioderivatives of Pyridine-2-carbonitrile

		Log P				Stationary Phase			
		Mathematical Method				Separon		LiChrospher	
Compound	R	Crippen[19]	Viswan.[18]	Broto[20]	HyperCh.[17]	k	Log k	k	Log k
N1	H	3.33	3.04	2.29	3.04	24.13	1.38	11.29	1.05
N2	2-Cl	3.88	3.55	2.91	3.55	55.44	1.74	22.71	1.36
N3	3-Cl	3.88	3.55	2.91	3.55	37.08	1.57	22.75	1.36
N4	4-Cl	3.88	3.55	2.91	3.55	42.94	1.63	25.47	1.41
N5	2-F	3.48	3.18	2.42	3.18	33.93	1.53	11.70	1.07
N6	3-F	3.48	3.18	2.42	3.18	20.79	1.32	11.31	1.05
N7	3-Br	4.15	3.83	3.17	3.83	52.54	1.72	28.44	1.45
N8	4-Br	4.15	3.83	3.17	3.83	85.03	1.93	29.30	1.47
N9	3-CH_3	3.81	3.50	2.70	3.50	53.15	1.73	24.85	1.40
N10	3-NO_2	2.62	2.86	0.93	2.99	14.36	1.16	7.68	0.89
N11	4-NO_2	2.62	2.86	0.93	2.99	15.06	1.18	7.77	0.89
N12	2-F,6-NO_2	2.78	3.00	1.07	3.13	17.97	1.26	8.36	0.92
N13	2-F,6-Cl	4.04	3.69	3.04	3.69	48.36	1.68	23.89	1.38
N14	4-OCH_3	3.20	**2.78**	2.42	**2.78**	27.13	**1.43**	13.42	**1.13**
N15	3,4-Cl	4.44	4.07	3.52	4.07	90.82	1.96	46.74	1.67
N16	3,4-F	3.64	3.32	2.56	3.32	22.21	1.35	14.66	1.17
N17	2,4-NO_2	**1.91**	2.68	**−0.42**	2.94	20.61	**1.31**	7.73	**0.89**

the series of 4-benzylthioderivatives of pyridine-2-carbothioamide, the values k and log k measured on the stationary phase Separon ranged between 17.84-174.44 and 1.25-2.24, respectively. On the stationary phase LiChrospher, these values for the same series ranged between 4.17-38.77 (k) and 0.86-1.56 (log k). In both types of stationary phases, the lowest values of the capacity factor were found by compound T18 (2,4-NO_2); on the other hand the highest retention was shown by compound T16 (3,4-Cl). For the series of 4-benzylthioderivatives of pyridine-2-carbonitrile, the values of k and log k measured on the stationary phase Separon ranged in the limits of 14.36-90.82 and 1.16-1.96, respectively. On the stationary phase LiChrospher, these values for the same series ranged from 7.68-46.74 (k) and 0.89-1.67 (log k). The lowest retention was shown by compound N10 (3-NO_2); on the other hand the highest retention was measured by compound N15 (3,4-Cl) on both types of the column.

The calculated values of log P were compared with the measured values of log k for both stationary phases. Good correlation was observed between log P and log k values. For the series of thioamide derivatives, the best correlation was found for the case when the values of log P obtained by the Viswanadhan method

Table 5. Deviating Compounds of the Series of 4-Benzylderivatives of Pyridine-2-carbothioamide

Deviating Compound	R	Log P	Mathematical Method	Log k	Stationary Phase	Correlation Coefficient r: Included in Correlation	Correlation Coefficient r: Excluded from Correlation
T1	H	2.74	Viswanadhan	2.05	Separon	0.850	0.928
		2.65	HyperChem	2.05	Separon	0.800	0.932
T15	4-OCH_3	2.49	Viswanadhan	1.13	LiChrospher	0.933	0.965
		2.40	HyperChem	1.13	LiChrospher	0.880	0.935
		2.40	HyperChem	1.58	Separon	0.800	0.932

and the values of log k measured on the stationary phase LiChrospher were mutually compared. Equation of correlation dependence: $\log P = 1.60 \log k + 1.10$; $n = 19$; $r = 0.933$; $s = 0.108$; $F = 107$. The dependence of log P on log k was demonstrated with a reliability of 99.9%.

For the series of nitrile derivatives, the best correlation was also found for the case when the values of log P obtained by the method of Viswanadhan and the values of log k measured on the stationary phase LiChrospher, were mutually compared. Equation of correlation dependence: $\log P = 1.60 \log k + 1.38$; $n = 17$; $r = 0.945$; $s = 0.140$; $F = 62.4$. The dependence of log P on log k was demonstrated with a reliability of 99.9%.

Table 6. Deviating Compounds of the Series of 4-Benzylthioderivatives of Pyridine-2-carbonitrile

Deviating Compound	R	Log P	Mathematical Method	Log k	Stationary Phase	Correlation Coefficient r: Included in Correlation	Correlation Coefficient r: Excluded from Correlation
N14	4-OCH_3	2.78	Viswanadhan	1.13	LiChrospher	0.945	0.977
		2.78	HyperChem	1.13	LiChrospher	0.919	0.971
		2.78	HyperChem	1.43	Separon	0.880	0.925
N17	2,4-NO_2	1.91	Crippen	0.89	LiChrospher	0.921	0.955
		1.91	Crippen	1.31	Separon	0.850	0.919
		−0.42	Broto	1.31	Separon	0.780	0.880
		−0.42	Broto	0.89	LiChrospher	0.870	0.913

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 thioamide derivatives, compounds T1 (unsubstituted) and T15 (4-OCH_3) markedly deviated in correlations. In the series of nitrile derivatives, compounds N14 (4-OCH_3) and N17 (2,4-NO_2) markedly deviated.

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