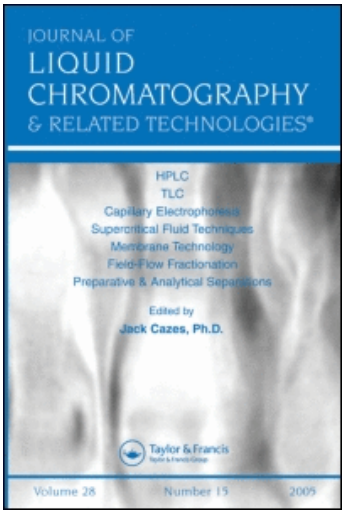

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Reversed-Phase High Performance Liquid Chromatographic Determination of Lipophilicity of Potential Antituberculosis Compounds

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ABSTRACT

Lipophilicity is one of the properties, which influences the partition of a substance in biological media. The reversed-phase high performance liquid chromatography (RP-HPLC) capacity factors k of 27 2-benzylsulfanyl derivatives of benzothiazole, 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 mathematical methods. High correlation was found between log P and log k values.

Key Words: Lipophilicity; RP-HPLC; Antituberculosis compounds; Octanol/water system.

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INTRODUCTION

Lipophilicity of a substance is one of the parameters, which plays a basic role in many biological processes. Lipophilicity is generally defined as the tendency of a chemical (drug) to distribute between an immiscible non-polar (organic) 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*) is widely used because of its simplicity and some similarity between *n*-octanol and biological membranes.[3] Nowadays, the determination of the partition coefficient usually measured by the "shake-flask" method, is often superseded by chromatographic methods. They are rapid and relatively simple, very small quantities of substances are required, and the compounds need not be very pure. In addition, by using the "shake-flask" method, the log *P* values are limited between -2 and $+4$.[4,5] Lipophilicity can be determined by reversed-phase high performance liquid chromatography (RP-HPLC)[3,6–11] and by reversed-phase thin-layer chromatography.[10,12–18] 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 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.[10,19–21] For hydrophilic compounds, this value can be measured directly, and it is considered to be related to log *P* values, as a measure of the lipophilic character of the substance.

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 SP8700 pump, a SP8750 sampler, and a UV detector Spectra 100 (all Spectra Physics, CA, USA).

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, 250 × 4 mm I.D., 5 μm (Merck, Germany).

Chemicals

The structures of the 27 2-benzylsulfanylbenzothiazole derivatives under examination are shown in Table 1. These compounds were previously synthesized as potential antituberculous drugs.[22] 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*

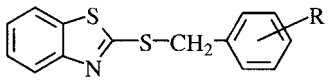
The mobile phases were made by mixing methanol with water in proportions 55 : 45, 60 : 40, 65 : 35, 70 : 30 (v/v). The optimal composition of the mobile phase for all tested compounds was methanol–water, (65 : 35)(v/v). The flow rate was 1 mL/min. A methanolic solution of potassium iodide was used for t_0 measurement. The measurements were done by using UV detection at 222 nm.

RESULTS AND DISCUSSION

RP-HPLC 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, Canada) and on the software HyperChem program Version 7.03 (HyperCube Inc., Gainesville, FL). Correlation and regression analysis of log P and log k were run on a PC computer using the Microsoft Excel program.

Table 2 sums up all results obtained from both RP-HPLC measurements, and by the calculation by means of the above mentioned program and methods for all tested compounds. The values k and log k measured on the stationary phase LiChrosphere ranged between 2.40–177.92 and

Table 1. Structure of 2-benzylsulfanylderivatives of benzothiazole.



Compound	R	Empirical formula	M_r
A1	2-SH-BTH[a]	$C_7H_5NS_2$	167.2
A2	H	$C_{14}H_{11}NS_2$	257.4
A3	4-Cl	$C_{14}H_{10}ClNS_2$	291.8
A4	2-Cl	$C_{14}H_{10}ClNS_2$	291.8
A5	4-F	$C_{14}H_{10}FNS_2$	275.4
A6	3-F	$C_{14}H_{10}FNS_2$	275.4
A7	4-Br	$C_{14}H_{10}BrNS_2$	336.3
A8	3-Br	$C_{14}H_{10}BrNS_2$	336.3
A9	4-CH_3	$C_{15}H_{13}NS_2$	271.4
A10	4-OCH_3	$C_{15}H_{13}NOS_2$	287.4
A11	3-OCH_3	$C_{15}H_{13}NOS_2$	287.4
A12	4-NO_2	$C_{14}H_{10}N_2O_2S_2$	302.4
A13	3-NO_2	$C_{14}H_{10}N_2O_2S_2$	302.4
A14	2-F–6-Cl	$C_{14}H_9ClFNS_2$	309.8
A15	3,4-Cl_2	$C_{14}H_9Cl_2NS_2$	326.3
A16	3,4-F_2	$C_{14}H_9F_2NS_2$	293.4
A17	3,5-$(NO_2)_2$	$C_{14}H_9N_3O_4S_2$	347.4
A18	2,4-$(NO_2)_2$	$C_{14}H_9N_3O_4S_2$	347.4
A19	2-F–6-NO_2	$C_{14}H_9FN_2O_2S_2$	320.4
A20	4-CF_3	$C_{15}H_{10}F_3NS_2$	325.4
A21	3-CF_3	$C_{15}H_{10}F_3NS_2$	325.4
A22	3,5-$(CF_3)_2$	$C_{16}H_9F_6NS_2$	393.4
A23	2-NO_2	$C_{14}H_{10}N_2O_2S_2$	302.4
A24	4-CN	$C_{15}H_{10}N_2S_2$	282.4
A25	3-CN	$C_{15}H_{10}N_2S_2$	282.4
A26	4-$CSNH_2$	$C_{15}H_{12}N_2S_3$	316.5
A27	3-$CSNH_2$	$C_{15}H_{12}N_2S_3$	316.5

[a]A1 is 2-sulfanylbenzothiazole without substitution.

0.38–2.25, respectively. The lowest values of the capacity factor were found by compounds A26 and A27 (4-$CSNH_2$ resp. 3-$CSNH_2$) (except A1, which is only 2-sulfanylbenzothiazole). On the other hand, the highest retention was shown by compound A22 (3,5-CF_3).

The calculated values of log P were compared with the measured values of log k. Good correlation was observed between log P and log k values. The dependence of log P was demonstrated with a reliability of 99.9%. Better

Table 2. log P, k and log k values of 2-benzylsulfanylderivatives of benzothiazole.

Compound	R	K	log k	log P (ACD/log P)	log P (HyperChem)
A1	2-SH-BTH[a]	2.40	0.38	3.31	2.31
A2	H	42.79	1.63	5.21	4.22
A3	4-Cl	82.40	1.92	5.81	4.73
A4	2-Cl	87.01	1.94	5.81	4.73
A5	4-F	43.39	1.64	5.27	4.36
A6	3-F	44.97	1.65	5.27	4.36
A7	4-Br	98.53	1.99	5.99	5.01
A8	3-Br	93.34	1.97	5.99	5.01
A9	4-CH_3	87.19	1.94	5.67	4.68
A10	4-OCH_3	46.76	1.67	5.13	3.96
A11	3-OCH_3	44.28	1.65	5.13	3.96
A12	4-NO_2	30.95	1.49	4.94	4.14
A13	3-NO_2	34.33	1.54	4.94	4.17
A14	2-F–6-Cl	80.67	1.91	5.42	4.87
A15	3,4-Cl_2	145.22	2.16	6.28	5.25
A16	3,4-F_2	52.76	1.72	5.23	4.49
A17	3,5-$(NO_2)_2$	35.09	1.55	4.61	4.12
A18	2,4-$(NO_2)_2$	36.64	1.56	4.61	4.12
A19	2-F–6-NO_2	34.03	1.53	4.90	4.31
A20	4-CF_3	89.89	1.95	5.79	5.10
A21	3-CF_3	74.41	1.87	5.79	5.10
A22	3,5-$(CF_3)_2$	177.92	2.25	6.56	5.98
A23	2-NO_2	31.58	1.50	4.94	4.17
A24	4-CN	17.31	1.24	4.65	4.25
A25	3-CN	18.10	1.26	4.65	4.25
A26	4-$CSNH_2$	10.44	1.02	4.49	3.87
A27	3-$CSNH_2$	9.57	0.98	4.49	3.87

[a]A1 is 2-sulfanylbenzothiazole without substitution.

correlation dependence for the series was achieved by using ACD/log P program, the equation of this dependence is:

$$\log P = 1.63 \log k + 2.57; \quad n = 27; r = 0.952; s = 0.212$$

The equation of the dependence log P on log k values by using a HyperChem program is:

$$\log P = 1.49 \log k + 2.00; \quad n = 27; r = 0.908; s = 0.280$$

In our previous papers,[9,11] we tested an influence of exclusion of some compounds, which deviated from correlation dependence. By exclusion of

the most "deviating" compounds, A26 (4-$CSNH_2$) and A27 (3-$CSNH_2$) was the correlation coefficient of regression dependence, improved from 0.908 to 0.922 (by using HyperChem program) and from 0.952 to 0.959 (by using ACD/log *P*). In contrast to our previous measurements, an improvement of correlation coefficients was not as strong as could be expected. The reason is probably because the computer programs used can calculate parameters of the lipophilicity with reasonable reliability of all tested compounds.

In general both programs are suitable for calculation log *P* values, although better correlation parameters were achieved by using ACD/log *P* program Version 1.0. The difference between experimental found values and calculated values could be caused by a method of calculation of log *P* values. In addition, when substitution of the ring is more complicated, the functional groups not taken into account by the computer program are most mutually influenced.

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