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Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 310–314
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Determination of lipophilicity of novel potential antituberculotic agents using HPLC on monolithic stationary phase and theoretical calculations

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Received 15 October 2007; received in revised form 17 December 2007; accepted 21 December 2007
Available online 4 January 2008

Abstract

The HPLC analyses on the monolithic stationary phase were employed for rapid determination of lipophilicity of the two sets of newly synthesized potential antituberculotic agents. The analyses utilized the mixture of methanol and phosphate buffer (pH 7.4) as a mobile phase and a flow rate of 4 mL/min. Monolithic stationary phase enabled to significantly reduce the time of analyses, achieve appropriate peak shapes for all tested compounds as well as the separation of positional isomers. Furthermore, the theoretical lipophilic parameters (log *P*) for all compounds were calculated employing the chemical programs (e.g., ACD/logP, HyperChem, miLogP, AlogP, KOWWIN and COSMOFrag, etc.). The experimental data (log *k*) and calculated log *P* values were compared by linear regression analysis. The highest correlation for both series was obtained for KOWWIN and miLogP programs. However, capability of particular chemical software to precisely predict lipophilicity of a compound is structurally dependent. Thus the predictive power of the selected program should be verified using experimental method. The results of this study documented that experimental determination of lipophilicity using HPLC on monolithic stationary phase is practical and reasonable for this purpose.
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Keywords: Lipophilicity; Monolithic column; HPLC; Partition coefficient; Chemical software

1. Introduction

At an early stage of drug discovery a large number of novel chemical entities pass through high-throughput screening in order to reveal their biological activity. Besides that, basic physico-chemical properties of the tested compounds are investigated as well. These may strongly contribute to the establishment of structure–activity relationships and/or to predict potential issues regarding the pharmacokinetics or pharmaceutical formulation of the novel drug candidates. Therefore, automatically feasible high-throughput determination of the selected physico-chemical characteristics can significantly accelerate the process of drug discovery and development.

Lipophilicity belongs to basic physico-chemical characteristics of a novel chemical entity. It significantly determines the behavior of a molecule in biphasic system. In biological systems lipophilicity largely determines the solubility of drugs in biological fluids, penetration through the biological membranes, rate of GIT absorption, affinity to plasma and tissue proteins, distribution in to the specific body compartments (e.g., CNS) or accumulation in organism, etc. [1–6]. Indeed, besides major impact on pharmacokinetics of novel drug candidates, lipophilicity also affects their pharmacodynamics. Therefore, determination of lipophilicity at the early stage of development can significantly limit the problems with poor ADME properties of novel drug candidates and/or to improve its efficacy, which certainly further underlines the importance of this parameter.

The basic experimental method for determination of lipophilicity is based on the partitioning of a molecule in a system of two immiscible phases (aqueous and lipophilic ones). Practically, this is performed using traditional shake-flask procedure with subsequent determination of the concentrations of the compound in both phases. Although different solvents were investigated for this purpose, octanol–water system remains the most popular model [6]. Using this approach lipophilicity of a compound is expressed as a logarithm of partitioning coefficient

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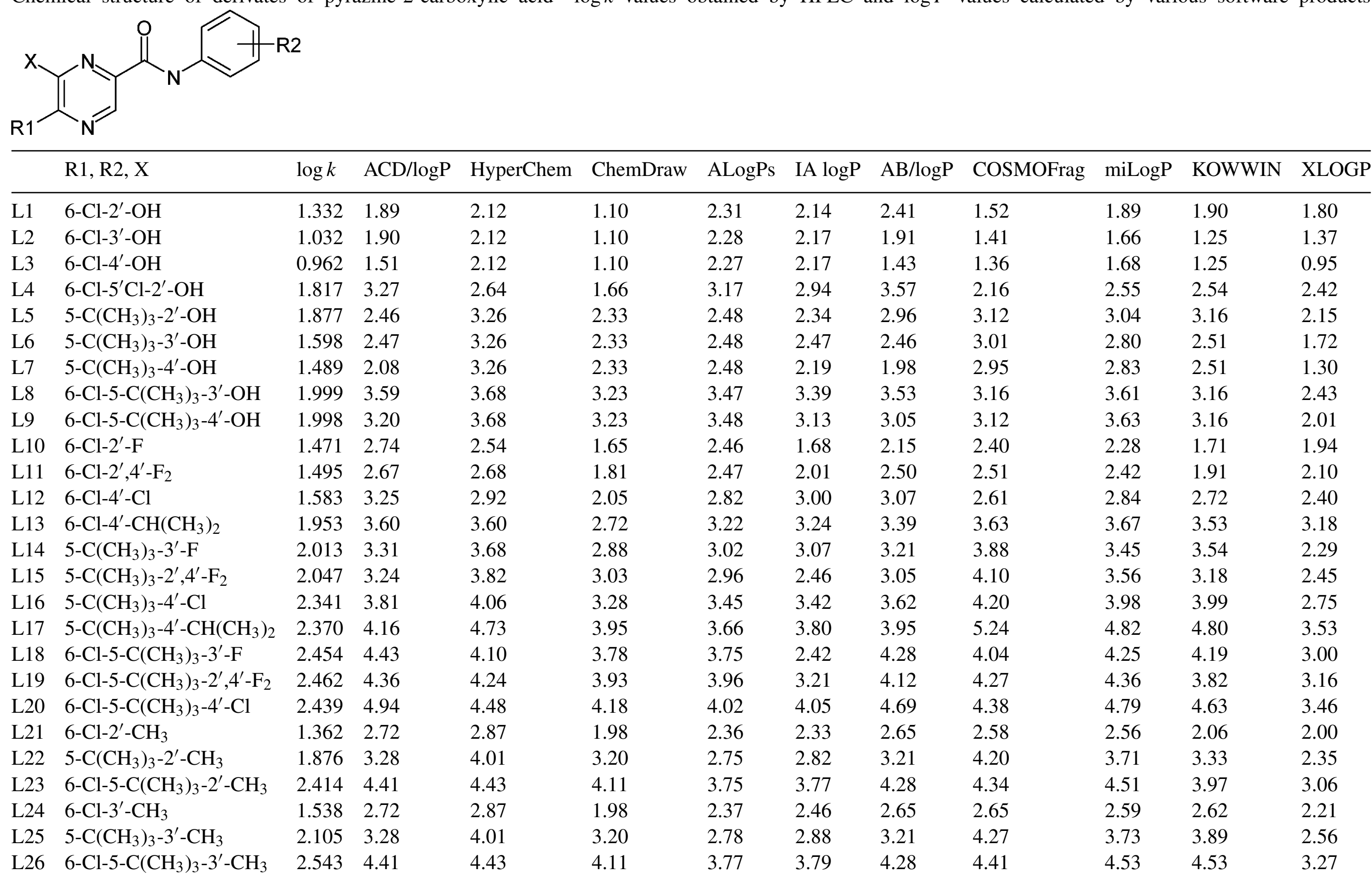
doi:10.1016/j.jpba.2007.12.040

Table 1
Classification of the software products utilized for prediction log P values in this study

Commercially available chemical software	
ACD/logP program Version 1.0 (Toronto, Canada)	Fragmental method
HyperChem program Version 7.03 (HyperCube Inc., Gainesville, Florida, U.S.A.)	Fragmental method
ChemDraw Ultra program Version 7.0 (CambridgeSoft, Cambridge, Massachusetts, U.S.A.) (ClogP)	Fragmental method
Programs available as a freeware	
IA logP	Whole molecular approach
COSMOFrag	Fragment contributions
miLogP	Fragment contributions
AB/logP	Atom/fragment contributions
AlogPs	Whole molecular approach
KOWWIN	Fragment contribution
XlogP	Atom contributions method

Table 2
Chemical structure of derivates of pyrazine-2-carboxylic acid—log k values obtained by HPLC and log P values calculated by various software products



	R1, R2, X	log k	ACD/logP	HyperChem	ChemDraw	ALogPs	IA logP	AB/logP	COSMOFrag	miLogP	KOWWIN	XLOGP
L1	6-Cl-2′-OH	1.332	1.89	2.12	1.10	2.31	2.14	2.41	1.52	1.89	1.90	1.80
L2	6-Cl-3′-OH	1.032	1.90	2.12	1.10	2.28	2.17	1.91	1.41	1.66	1.25	1.37
L3	6-Cl-4′-OH	0.962	1.51	2.12	1.10	2.27	2.17	1.43	1.36	1.68	1.25	0.95
L4	6-Cl-5′Cl-2′-OH	1.817	3.27	2.64	1.66	3.17	2.94	3.57	2.16	2.55	2.54	2.42
L5	5-C(CH_3)$_3$-2′-OH	1.877	2.46	3.26	2.33	2.48	2.34	2.96	3.12	3.04	3.16	2.15
L6	5-C(CH_3)$_3$-3′-OH	1.598	2.47	3.26	2.33	2.48	2.47	2.46	3.01	2.80	2.51	1.72
L7	5-C(CH_3)$_3$-4′-OH	1.489	2.08	3.26	2.33	2.48	2.19	1.98	2.95	2.83	2.51	1.30
L8	6-Cl-5-C(CH_3)$_3$-3′-OH	1.999	3.59	3.68	3.23	3.47	3.39	3.53	3.16	3.61	3.16	2.43
L9	6-Cl-5-C(CH_3)$_3$-4′-OH	1.998	3.20	3.68	3.23	3.48	3.13	3.05	3.12	3.63	3.16	2.01
L10	6-Cl-2′-F	1.471	2.74	2.54	1.65	2.46	1.68	2.15	2.40	2.28	1.71	1.94
L11	6-Cl-2′,4′-F_2	1.495	2.67	2.68	1.81	2.47	2.01	2.50	2.51	2.42	1.91	2.10
L12	6-Cl-4′-Cl	1.583	3.25	2.92	2.05	2.82	3.00	3.07	2.61	2.84	2.72	2.40
L13	6-Cl-4′-CH(CH_3)$_2$	1.953	3.60	3.60	2.72	3.22	3.24	3.39	3.63	3.67	3.53	3.18
L14	5-C(CH_3)$_3$-3′-F	2.013	3.31	3.68	2.88	3.02	3.07	3.21	3.88	3.45	3.54	2.29
L15	5-C(CH_3)$_3$-2′,4′-F_2	2.047	3.24	3.82	3.03	2.96	2.46	3.05	4.10	3.56	3.18	2.45
L16	5-C(CH_3)$_3$-4′-Cl	2.341	3.81	4.06	3.28	3.45	3.42	3.62	4.20	3.98	3.99	2.75
L17	5-C(CH_3)$_3$-4′-CH(CH_3)$_2$	2.370	4.16	4.73	3.95	3.66	3.80	3.95	5.24	4.82	4.80	3.53
L18	6-Cl-5-C(CH_3)$_3$-3′-F	2.454	4.43	4.10	3.78	3.75	2.42	4.28	4.04	4.25	4.19	3.00
L19	6-Cl-5-C(CH_3)$_3$-2′,4′-F_2	2.462	4.36	4.24	3.93	3.96	3.21	4.12	4.27	4.36	3.82	3.16
L20	6-Cl-5-C(CH_3)$_3$-4′-Cl	2.439	4.94	4.48	4.18	4.02	4.05	4.69	4.38	4.79	4.63	3.46
L21	6-Cl-2′-CH_3	1.362	2.72	2.87	1.98	2.36	2.33	2.65	2.58	2.56	2.06	2.00
L22	5-C(CH_3)$_3$-2′-CH_3	1.876	3.28	4.01	3.20	2.75	2.82	3.21	4.20	3.71	3.33	2.35
L23	6-Cl-5-C(CH_3)$_3$-2′-CH_3	2.414	4.41	4.43	4.11	3.75	3.77	4.28	4.34	4.51	3.97	3.06
L24	6-Cl-3′-CH_3	1.538	2.72	2.87	1.98	2.37	2.46	2.65	2.65	2.59	2.62	2.21
L25	5-C(CH_3)$_3$-3′-CH_3	2.105	3.28	4.01	3.20	2.78	2.88	3.21	4.27	3.73	3.89	2.56
L26	6-Cl-5-C(CH_3)$_3$-3′-CH_3	2.543	4.41	4.43	4.11	3.77	3.79	4.28	4.41	4.53	4.53	3.27

(log P) obtained in the octanol–water system. Unfortunately, shake-flask method is extremely time-consuming and labor-intensive, it requires a high purity of tested substances and often suffers from solubility and stability problems. Hence, nowadays this complicated approach was nearly completely substituted by modern chromatographic techniques [6–12].

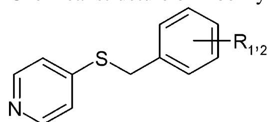
Among them, HPLC is the leading and the most frequently used chromatographic method for the routine lipophilicity determination, since it enables rapid, accurate and highly reproducibility analysis of relatively large sets of samples. These experiments are usually performed on reverse phase systems, where the chromatographic retention behaviour of an analyte is directly related to its lipophilicity. Employing HPLC the lipophilicity of a compound is expressed as a logarithm of retention factor (log k), which is calculated according to Eq. (1).

$$k = \frac{t_r - t_0}{t_0} \qquad (1)$$

where t_r is the retention time and t_0 is the dead retention time.

Nowadays, majority of these analyses utilize the common particle based stationary phases (C18 or C8). Since usually the analysis covers large sets of samples, the common particle based stationary phase may be inconvenient and impractical with regards on time required for analysis. Particularly, this could

Table 3
Chemical structure of 4-benzylsulfanilpyridine—log *k* values obtained by HPLC and log *P* values calculated by various software products



	R_1, R_2	log *k*	ACD/logP	HyperChem	ChemDraw	ALogPs	IA logP	AB/logP	COSMOFrag	miLogP	KOWWIN	XLOGP
3120	H	1.522	2.85	3.53	2.87	3.22	3.06	3.06	3.11	2.68	3.11	2.96
3122	4-F	1.561	2.90	3.67	3.03	3.13	3.28	3.28	3.26	2.84	3.31	3.12
3123	4-CF_3	2.083	3.42	4.42	3.79	3.46	4.15	4.15	3.85	3.57	4.08	3.88
3124	4-CN	1.026	3.42	3.57	2.90	2.79	2.75	2.75	2.36	2.43	2.66	2.68
3125	4-Cl	1.940	3.44	4.05	3.43	3.75	3.73	3.73	3.54	3.36	3.76	3.58
3126	4-CH_3	1.962	3.31	4.00	3.36	3.57	3.39	3.39	3.73	3.13	3.66	3.39
3127	3-CH_3	1.924	3.31	4.00	3.36	3.57	3.36	3.36	3.73	3.10	3.66	3.39
3128	3-Cl	1.898	3.44	4.05	3.43	3.74	3.71	3.71	3.65	3.33	3.76	3.58
3129	3-OCH_3	1.556	2.76	3.28	2.74	3.28	2.99	2.99	3.38	2.71	3.19	2.87
3130	3-F·HCl	1.533	2.76	3.67	3.03	3.11	3.21	3.21	3.39	2.82	3.31	3.12
3131	3-CF_3	2.017	3.42	4.42	3.79	3.49	4.07	4.07	3.80	3.55	4.08	3.88
3132	3-CN	1.020	2.29	3.57	2.90	2.76	2.71	2.71	2.47	2.41	2.66	2.68
3133	3-NO_2	1.240	2.58	3.49	2.51	2.96	2.20	2.20	2.99	2.61	2.93	2.85
3134	4-Br	2.055	3.62	4.32	3.70	4.02	3.76	3.76	3.81	3.49	4.00	3.75
3135	3-Br	1.996	3.62	4.32	3.70	4.01	3.75	3.75	3.85	3.46	4.00	3.75
3136	4-NO_2	1.251	2.58	3.49	2.51	2.96	2.30	2.30	2.96	2.64	2.93	2.85
3137	4-OCH_3	1.587	2.76	3.28	2.74	3.30	3.03	3.03	3.30	2.73	3.19	2.87
3138	2-Cl	1.899	3.44	4.05	3.43	3.72	3.71	3.71	3.74	3.31	3.76	3.58
3139	2-F·HCl	1.572	2.90	3.67	3.03	3.08	3.11	3.11	3.34	2.79	3.31	3.12
3140	2-NO_2	1.206	2.58	3.49	2.51	2.84	2.08	2.80	3.27	2.59	2.93	2.85
3141	3,5-$(NO_2)_2$	1.153	2.25	3.44	2.47	2.73	1.50	1.50	3.55	2.52	2.75	2.74
3142	3,4-Cl_2	2.293	3.91	4.57	3.99	4.27	4.45	4.45	4.11	3.96	4.40	4.20
3143	3,5-(CF_3)	2.509	4.19	5.30	4.71	4.34	4.89	4.89	4.83	4.39	5.04	4.81
3144	2-Cl-6-F	1.924	3.05	4.19	3.59	3.72	3.74	3.74	3.82	3.42	3.96	3.74
3145	2-F-6-NO_2	1.285	2.53	3.63	2.64	2.99	2.04	2.04	3.39	2.70	3.13	3.01
3146	4-$CSNH_2$	0.896	2.12	3.19	2.34	2.72	2.69	2.69	2.17	2.48	2.79	2.09
3147	3-$CSNH_2$	0.894	2.12	3.19	2.34	2.69	2.69	2.69	2.28	2.45	2.79	2.09
3148	2.4-(NO_2)	1.213	2.25	3.44	2.47	2.60	1.29	1.29	3.51	2.50	2.75	2.74

be an issue in the case of highly lipophilic samples which are strongly retained on RP stationary phase, where the conventional HPLC analyses are extremely time-consuming. From that point of view, modern monolithic stationary phases seem to be a reasonable alternative to the common stationary phase. Their higher permeability and higher stability resulted from the nature of monoliths enable to use fast flow. The application of high flow rate allows overcome issues given above, improve the separation, peak shapes and mainly significantly reduce the time of analysis. On the other hand, the higher solvents consumption is a certain limitation of this approach [13–15].

Apart from the experimental methods, the lipophilicity of novel drug candidates can be estimated using various chemical software products based on the different mathematical models. The most frequently used mathematical models are based on either the substructure or whole molecular approaches. The first one predicts log *P* according to the contributions of each molecular fragments, while the second utilizes the molecular

Table 4
Parameters and statistical data of regression equations of correlation between log *k* and log *P* values calculated by chemical software in the group of derivates of pyrazine-2-carboxylic acid (software products are in order of decreasing correlation level)

	n	Slope	Intercept	*R*	*s*
miLogP	26	1.995 (±0.116)	−0.429 (±0.223)	0.962	0.264
KOWWIN	26	2.135 (±0.130)	−0.917 (±0.250)	0.958	0.297
ChemDraw	26	2.059 (±0.143)	−1.145 (±0.275)	0.947	0.325
AB/logP	26	1.714 (±0.129)	−0.063 (±0.248)	0.938	0.294
HyperChem	26	1.615 (±0.125)	0.429 (±0,240)	0.935	0.284
ACD/logP	26	1.833 (±0.145)	−0.204 (±0.279)	0.932	0.331
ALogPs	26	1.180 (±0.110)	0.795 (±0.212)	0.909	0.251
COSMOFrag	26	2.085 (±0.198)	−0.605 (±0.379)	0.907	0.449
XLOGP	26	1.313 (±0.136)	−0.074 (±0.260)	0.892	0.308
IA logP	26	1.097 (±0.179)	0.771 (±0.344)	0.781	0.407

n is the number of substances, *R* is the correlation coefficient, *s* is the residual sum of squares.

Table 5
Parameters and statistical data of regression equations of correlation between log k and log P values calculated by chemical software in the group of 4-benzylsulfanylpyridine (software products are in order of decreasing correlation level)

	n	Slope	Intercept	R	s
KOWWIN	28	1.371 (±0.070)	1.307 (±0.117)	0.965	0.161
XLOGP	28	1.323 (±0.079)	1.093 (±0.132)	0.957	0.181
miLogP	28	1.104 (±0.072)	1.224 (±0.120)	0.949	0.165
ALogPs	28	1.075 (±0.070)	1.587 (±0.117)	0.949	0.161
ChemDraw	28	1.245 (±0.095)	1.117 (±0.159)	0.932	0.218
COSMOFrag	28	1.183 (±0.115)	1.498 (±0.191)	0.897	0.262
HyperChem	28	1.007 (±0.098)	0.164 (±0,164)	0.895	0.225
ACD/logP	28	1.137 (± 0.111)	1.166 (±0.185)	0.894	0.255
IA logP	28	1.670 (±0.194)	0.445 (±0.322)	0.861	0.443
AB/logP	28	1.615 (±0.192)	0.559 (±0.320)	0.855	0.440

n is the number of substances, R is the correlation coefficient, s is the residual sum of squares.

characteristics such as molecular lipophilicity potentials, molecular properties (e.g., volume weight, molecular surface area) or topological indices. Nevertheless, the molecular approach is generally preferred since it takes into account steric and conformation effects and therefore it is able to distinguish the structural isomers and overall it considers the whole molecule as a complex [16,17].

Despite the fact that the theoretical calculations represent a certain alternative to the experimental methods, the routine application of this approach definitely requires a comparison of their results with the data obtained using experimental methods.

The aim of this study was to employ fast HPLC analyses on the monolithic column to determine the lipophilicity of two series of potential antituberculotic agents (anilides of pyrazine-2-carboxylic acid and derivates of 4-benzylsulfanylpyridine). Subsequently, these parameters (log k) were compared with the log P values estimated using different software products.

2. Experimental

2.1. Drugs and chemicals

Analyzed compounds were synthesized in-house at the Faculty of Pharmacy, Charles University in Prague. Their structures and purity were approved by NMR [18–22]. The compounds belonging to the first series (anilides of pyrazine-2-carboxylic acid, Table 2) are marked as L1–L26 and the derivates of 4-benzylsulfanylpyridine (Table 3) are labeled as 3120–3148. Methanol, potassium dihydrogen phosphate and potassium hydroxide were purchased from Penta (Prague, Czech Republic). The water was prepared by Milli-Q® Ultra-pure Water Purification System (Millipore).

2.2. Lipophilicity determination

2.2.1. HPLC analysis

The analyses of all compounds were carried out on a monolithic chromatographic column Chromolith RP18e; 100 mm × 4.6 mm (Merck, Darmstadt, Germany). The chromatographic system LC 20A (Shimadzu, Duisburg, Germany) consisted of a DGU-20A3 degasser, two LC-20 AD pumps, a SIL-20 AC autosampler, a CTO-20AC column oven, a SPD M10AVP UV/VIS detector and a CBM-20AC communication module was used for the analyses. The chromatographic data were processed using LC solution software, version 1.21 SP1 (Schimadzu, Duisburg, Germany). The column oven was set at 30 °C. UV detection was performed at 254 nm. An injection volume of 100 μL was used in the analyses. Each of the samples was injected onto a column in duplicate. The methanol and 0.05 M phosphate buffer (pH 7.4) in ratio 42:58 (v/v) for analysis of anilides of pyrazin-2-carboxylic acid or 37:63 (v/v) for analysis of derivates of 4-benzylsulfanylpyridine were employed as mobile phases. The flow rate was 4.0 mL/min.

Stock solutions of each compound were prepared in methanol (2.0 mg/mL). The analyzed solutions were obtained by appropriate dilution of the stock solutions with mobile phase to get a concentration of 0.5 mg/mL for each compound.

A methanolic solution of potassium iodide was used to determine the dead retention time (t_0). The retention factors were calculated according to Eq. (1).

2.2.2. Calculation log P using the chemical software

In order to estimate log P values of the tested compounds following software products were employed: ACD/logP, HyperChem, ChemDraw Ultra and AlogPs, IA logP, AB/logP, COSMOFrag, miLogP, KOWWIN and XLOGP available as a freeware [23]. The programs used in this study are summarized in Table 1. The experimental (log k) and predicted data (log P) were compared by regression analysis.

3. Results and discussion

The lipophilicity of the two series of potential antituberculotic agents was determined using HPLC analyses on the monolithic stationary phase (Chromolith RP18e). This particular stationary phase was chosen to reduce the time of analysis which was expected to be very long on the conventional particle-based columns mainly with regard to the rather higher lipophilicity of these samples. Moreover, this approach enables to get symmetrical peaks even in the case of the most lipophilic compounds of the series as well as to reach sufficient separation of the positional isomers in relatively short time. Before analyses, compositions

of mobile phases were optimised in order to achieve the sufficient separation of all the tested compounds. The composition of mobile phases was chosen according to results of the pilot analyses in which the least and the highest lipophilic compounds of each series were analyzed. According to their retention and resolution the highest content of organic solvent which enabled to get acceptable resolution and retention of all analytes was selected for each series. The pH of 7.4 was selected to meet the physiologically relevant conditions.

The $\log k$ calculated from the retention times of the compounds and the dead time of the chromatographic system are given in Tables 2 and 3. The halogenated derivates with isobutyl and isopropyl substituents were determined as the most lipophilic compounds in both groups.

Beside $\log k$ values assessed using HPLC; the $\log P$ values were calculated using different software products employing both the fragmental and the whole molecule approaches. Results of these calculations are shown in Tables 2–3. Results of statistical comparison of $\log k$ and $\log P$ values are given in Tables 4 and 5. It is obvious that the majority of tested software products provided the results that were in good correlation with the parameters determined experimentally (R values were ranged from 0.97 to 0.85). Interestingly, it was possible to find two programs (KOWWIN and miLogP) which gave nearly similar and relatively high correlations for both structurally different groups under the study. Both utilized the principle of the fragmental approach. Hence, based on these results, we can consider that these programs are the most convenient and they appear to be universal, at least to a certain degree, for the calculation of the lipophilicity in the both tested series.

On the other hand, distinct results were obtained with XlogP. Although XlogP utilized the same calculation model as both products mentioned above, it is suitable for the lipophilicity prediction only in the case of 4-benzylsulfanylpyrazines ($R = 0.957$). In the series of pyrazin-2-carboxylic acid derivates its predictive power failed ($R = 0.892$). These results underlined the importance of the proper selection of the particular program to theoretically assess the lipophilicity of the drugs.

Although the good correlation between the $\log k$ determined chromatographically and the $\log P$ values calculated using the selected chemical programs (especially, KOWWIN and miLogP) were obtained, prior routine application of this approach, it is necessary to experimentally verify the predictive power of particular product to lipophilicity determination in the specific series of the tested compounds. Importantly, our results suggest that HPLC analyses using monolithic based stationary phase might be a practical and convenient option.

4. Conclusion

In this study lipophilic parameters of two series of newly synthesized antituberculotic compounds were determined using both experimental method (RP-HPLC) and the theoretical calculations. The employment of monolithic stationary phase reduced the time of analyses and let us to obtain well separated and symmetrical peaks even in the case of strongly retained compounds and/or positional isomers. Correlations between $\log k$ values determined by RP-HPLC and $\log P$ predicted using number of chemical software pointed out that KOWWIN and miLogP as the most useful programs for the lipophilicity prediction in both structurally different series of compounds. Since capability of particular chemical software to accurately predict lipophilicity of a compound is strongly influenced by its chemical structure, the predictive power of the selected program should be verified using experimental methods. The results of the present study documented that experimental determination of lipophilicity using HPLC with monolithic based stationary phase is practical and reasonable option which is particularly advisable in the analysis of large sets of samples of higher lipophilicity. In addition, the outcomes of this study provided information that could be utilized in further development of novel derivatives of antituberculotic agents.

Acknowledgement

This work was supported by research project MSM 0021620822.

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