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REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY STUDY OF LIPOPHILICITY OF IMIDAZO[2,1-*F*]THEOPHYLLINE DERIVATIVES

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Abstract: The present study is a part of our physicochemical and pharmacological studies in a group of tricyclic theophylline derivatives. The investigated compounds exhibit different pharmacological profiles in comparison to theophylline and have been tested as potential antidepressant and/or antipsychotic agents. The differences in pharmacological action between theophylline and their tricyclic derivatives can be explained by their various physicochemical properties, especially lipophilicity. The chromatographic behavior of twenty three derivatives of imidazo[2,1-*f*]theophylline was investigated, using reversed-phase high performance liquid chromatography (RP-HPLC) method. Moreover, partition coefficients and selected pharmacokinetic parameters were calculated computationally. Principal component analysis (PCA) method was used to establish the relationship between obtained experimental and computational parameters.

Keywords: tricyclic theophylline derivatives, lipophilicity, logP

Theophylline - discovered in the nineteen thirties - is a measure commonly used in the treatment of bronchial asthma and other respiratory diseases; diseases caused by airways obstruction. The observed effects of theophylline are the result of a number of biochemical mechanisms such as the binding towards adenosine receptors, non-selective inhibition of the various phosphodiesterases (PDE), increasing the supply of intracellular calcium ions and indirect effects on different biochemical and enzymatic pathways (1, 2). Theophylline has a strong pharmacological effect on central nervous system (CNS) that causes the improvement of associative processes, suppression of fatigue and drowsiness, stimulates the sensory centers, including the respiratory center. Theophylline works as psychostimulant but its influence is weaker than the influence of caffeine as penetration of the brain through the blood-brain barrier is weaker as well. Theophylline is rapidly and completely absorbed after oral administration in solution or immediate-release solid oral dosage form. The estimation of pharmacokinetic of theophylline shows that nearly 60% is binding to human plasma proteins, primarily to albumin. Disadvantages of theophylline in the treatment are of a small range between therapeutic and toxic dose and the dependence of blood concentrations of creatinine clearance. The observed side effects depend on the concentration of theophylline in the blood and manifest themselves as headache, nausea, vomiting, insomnia, cardiac arrhythmia, in the case of acute poisoning - convulsions and cardiac arrest (3). Modifications of the structure of theophylline, which can be seen as a pharmacophore, lead to compounds with targeted pharmacological effect and/or with improved physicochemical properties, especially solubility, which has a decisive influence on the pharmacokinetics and bioavailability.

Studies carried out on the synthesis and determination of pharmacological properties of new tricyclic theophylline derivatives, showed that introduction in 7,8-position of theophylline five or six membered, heterocyclic rings (imidazole or pyrimidine) caused the change of the action profile of these compounds in the CNS compared to the activity of the theophylline (4, 5). For instance, derivatives of imidazo[2,1-*f*]purine-2,4-dione showed high *in vitro*

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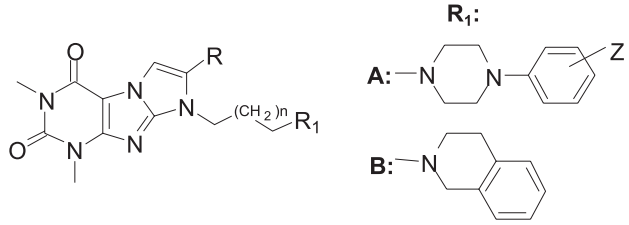
activity at 5-HT_{1A} receptors and its intrinsic *in vivo* activity at this receptor subtype was diversified (6, 7). Preclinical studies indicated that some of compounds exerted anxiolytic-like activity in the four-plate test on mice and behaved like antidepressants in the forced swimming test on mice. The results suggest that tricyclic derivatives of theophylline, especially with the long chain arylpiperazines (LCAPs) substituent, remain a worthy and interesting field for future research to obtain new compounds with potential anxiolytic/antidepressant activity.

In medicinal chemistry numerous measured or calculated parameters have been used for characterization of the lipophilicity but the logarithm of the n-octanol/water partition coefficient (logP) is still generally accepted and primarily applied descriptor of the lipophilicity in quantitative structure-activity relationships (QSAR) studies. Lipophilicity of drug candidate can affect both the pharmacokinetic and pharmacodynamic properties. In particular, the ability of a molecule to cross the cell membrane depends on its partition coefficient. Transport across the blood-brain barrier (BBB) is the most important feature of new drugs intended to be active in the central nervous system (8).

In the paper, we describe the estimation of the lipophilicity of some LCAPs and tetrahydroisoquinoline (THIQ) derivatives with imidazo[2,1-*f*]purine-2,4-dione moiety (**1**-**23**) (9) by using reversed-phase high-performance liquid chromatography (RP-HPLC) method. It shows that pharmacological profile of compounds **1**-**23** can be explained by different features than theophylline physicochemical properties. The relationship between the concentration of the organic modifier in the mobile phase and the chromatographic properties of the investigated compounds; as well as the influence of substituent on the lipophilicity of compounds, were studied. Moreover, partition coefficients values were obtained using software packages. Next step of investigation was to establish the relationship between $\log k_0$ and theoretically calculated parameters using principal component analysis (PCA) method. For analysis of pharmacokinetic properties, the selected absorption, distribution, metabolism or elimination (ADME) parameters were obtained by the use of computational methods. The relationships between $\log k_0$ and values of passive diffusion/permeability (LogPS), brain penetration (LogBB), passive absorption (PA%), and plasma protein binding (PB%) were establish by PCA method.

Table 1. The structures of compounds **1**-**23**.



Comp	R	R_1	n	Z	Comp	R	R_1	n	Z
1	H	A	1	H	**13**	C_6H_5	A	1	3-Cl
2	H	A	1	2-OCH_3	**14**	H	A	2	H
3	H	A	1	3-Cl	**15**	H	A	2	2-OCH_3
4	H	A	1	2,3-diCl	**16**	H	A	2	3-Cl
5	H	B	1	-	**17**	H	B	2	-
6	CH_3	A	1	H	**18**	CH_3	A	2	H
7	CH_3	A	1	2-OCH_3	**19**	CH_3	A	2	2-OCH_3
8	CH_3	A	1	3-Cl	**20**	CH_3	A	2	3-Cl
9	CH_3	A	1	2,3-diCl	**21**	CH_3	B	2	
10	CH_3	B	1	-	**22**	C_6H_5	A	2	H
11	C_6H_5	A	1	H	**23**	C_6H_5	B	2	-
12	C_6H_5	A	1	2-OCH_3					

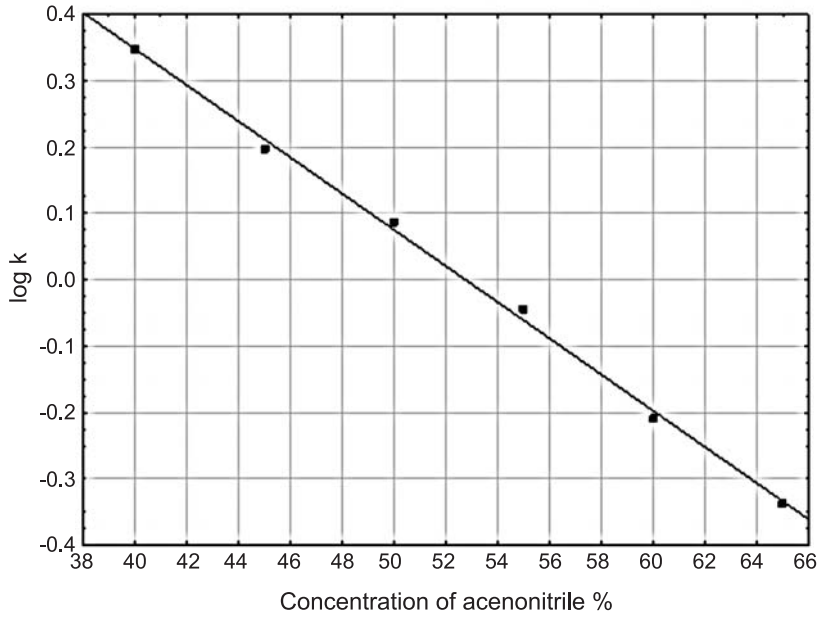


Figure 1. The dependence between log k values and concentration of acetonitrile in mobile phase for compound **12**

EXPERIMENTAL

Materials and methods

Analytical grade chemicals were used, unless indicated otherwise. Acetonitrile, water (HPLC grade) and trifluoroacetic acid (TFA) were supplied by Merck (Darmstad, Germany). Water was deionized by use of a Milipore system. The investigated LCAPs and THIQ derivatives of imidazo[2,1-*f*]purine-2,4-dione were synthesized according to the methods described in the literature (6, 7). The chemical structures of these compounds are presented in Table 1.

Chromatography

RP-HPLC analysis was performed on a Waters Alliance HPLC instrument with Waters 2695 Separation Module with photodiode array detector Waters 2998, absorbance was measured at wavelength 230 nm. Separation was performed on a C_{18} HPLC column (LiChrospher 100 R, 100 × 4.6 mm, 5 µm, Merck) fitted with a C_{18} guard column. A constant flow rate of 1 mL/min was used, the injection volume was 10 µL. Mobile phase A consisted of 0.01% trifluoroacetic acid (TFA) in water and mobile phase B consisted of 0.01% TFA in acetonitrile. Elution was isocratic with acetonitrile content between 65% and 35% (v/v) in 5% increments. The compounds were dissolved in methanol (0.5 mg/mL) and 10 µL solutions were separately injected on the column. The dead time was measured by injection of methanol. The retention times (t_r) and dead times (t_0) were the means of three independent determinations.

The isocratic capacity factors log*k*, were calculated from the experimental, retention times (t_r) and dead time (t_0) according to Eq. (1):

$$\log k = \log[(t_r - t_0)/t_0] \quad (1)$$

The log *k* values were extrapolated to zero acetonitrile concentration by use of equation 2:

$$\log k = \log k_0 - S\Phi \quad (2)$$

where, Φ is the concentration of acetonitrile in the mobile phase [*v*/*v*].

Calculations

The coefficients *A*logPs, milogP, $\log P_{KOWIN}$ and *X*logP2 were calculated from the Virtual Computational Laboratory website (10), $\log P_{Pallas}$ by Pallas 3.1 and XlogP by CAChe 7.75. ADME properties were prepared with ACD/Percepta trial version Build 2254 software. The SMILES codes were drawn in ACD/Labs.

Bartlett's test was performed, which verifies the null hypothesis that the correlation matrix is the identity matrix (coefficients between variables are zero). The coefficient of Kaiser-Mayer-Olkin (KMO) was also calculated, to verify the degree of correlation of the original variables. Before PCA has

been started, were evaluated the merits of its application. The resulting value of the statistics U Bartlet was 145.5 and the associated level of $p < 0.000001$, could rejected the null hypothesis. The resulting ratio for coefficient of KMO test was more than the average - 0.65. It was concluded that there was sufficient grounds for the use of principal components analysis (11).

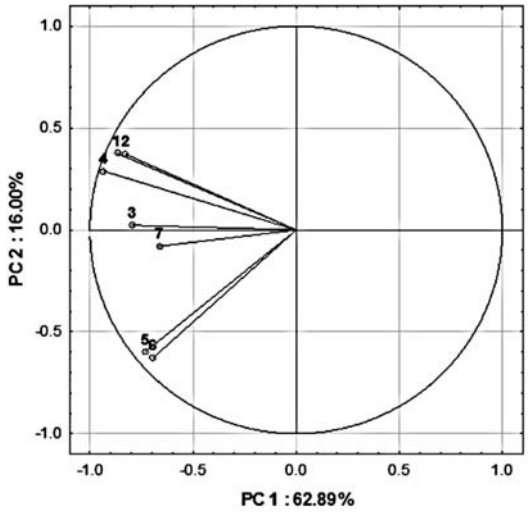


Figure 2. Comparison of the logk_0 and calculated coefficients by PCA. **1** - *A*logPs; **2** - milogP; **3** - $logP_{KOWIN}$; **4** - *X*logP2; **5** - $logP_{Pallas}$; **6** - $logP_{CAChe}$; **7** - logk_0. Vector log k_0 lies in the second quarter, shows a positive correlation with the third component, a negative correlation with the first component. It also has the highest values of the vector coordinates of the end as high (about 0.7) for the first and third component

All graphs and regression analysis was performed with the Statistica program (Statistica version 10.0, StatSoft Inc., 2011). The significance level of performed calculations was above 95%.

RESULTS

The retention factors log*k* of the compounds decreased linearly with increasing concentration of acetonitrile in the mobile phase. The obtained results enabled calculation of polycratic logk_0 values with equation (2). The dependence of the log*k* value on the concentration of acetonitrile for compound **12** is presented in Figure 1. The relative lipophilicity, expressed as logk_0 values and parameters obtained from the regression analysis, such as the correlation coefficients (*r*), the standard errors of estimation (*s*) and values of *F*-test of significance (*F*), are listed in Table 2. The significance level of performed calculation was $p \leq 0.05$

The linear correlation between the volume fraction of acetonitrile and the log*k* values was established with moderately low values of correlation coefficients ($0.9072 < r < 0.9785$) for compounds **1**, **2**, **3**, **5**, **10**, **15**, **21**, **23** and high for other ($r > 0.9815$). The relative lipophilicity of the investigated compounds (**1**-**23**) was low, the lowest for compound **5** (logk_0 = 0.217) and the highest for compound **13** (logk_0 = 1.339). The logk_0 values were also compared with the theoretical values of partition coefficients (Table 3). Noteworthy there is the large difference between the values set calculated by the computer programs and obtained experimental-

Table 2. Data for linear correlation (equation 2) between log*k* values and the concentration of acetonitrile [*C* (%, v/v)] in the mobile phase for compound **1**-**23**.

Compound	logk_0	*r*	*s*	*F*	n	Compound	logk_0	*r*	*s*	*F*	n
1	0.485	0.9106	0.0986	20	7	**13**	1.339	0.9718	0.0401	105	6
2	0.597	0.9354	0.0736	20	6	**14**	0.475	0.9624	0.0281	128	7
3	0.697	0.9371	0.0425	59	6	**15**	0.513	0.9251	0.0218	69	6
4	0.876	0.9869	0.0614	31	6	**16**	0.529	0.9577	0.0327	90	6
5	0.217	0.9412	0.0114	45	7	**17**	0.260	0.9611	0.0168	24	6
6	0.266	0.9815	0.0370	17	7	**18**	0.716	0.9860	0.0243	280	6
7	0.271	0.9950	0.0517	34	7	**19**	0.687	0.9764	0.0309	165	6
8	0.708	0.9642	0.0309	107	7	**20**	0.787	0.9785	0.0245	182	6
9	0.312	0.9686	0.0079	62	5	**21**	0.492	0.9279	0.0181	64	7
10	0.370	0.9072	0.0614	20	7	**22**	0.733	0.9868	0.0581	72	6
11	0.715	0.9764	0.0268	206	7	**23**	0.707	0.9450	0.0186	85	7
12	1.140	0.9971	0.0005	135	6	Theophylline	-0.95	0.9529	0.0491	87	6

Correlation coefficient (*r*), the standard error of estimation (*s*) and value of *F*-test of significance (*F*), n - number of data points.

Table 3. Six logP values obtained by the use of computational methods.

Compound	*A*logP$_s$	MlogP	logP$_{KOWIN}$	*X*logP2	logP$_{PALLAS}$	logP$_{CAChe}$
1	2.16	2.12	2.65	2.02	1.50	1.96
2	2.41	2.11	2.71	1.91	1.57	1.70
3	2.95	2.97	1.29	2.64	2.24	2.48
4	2.01	2.12	2.85	1.76	0.77	1.76
5	2.71	2.54	1.20	2.48	0.99	1.52
6	2.80	2.55	1.28	2.19	1.05	1.27
7	1.26	1.19	1.84	1.10	1.71	2.04
8	2.41	2.14	2.40	2.22	1.28	1.11
9	1.65	1.99	4.41	1.81	2.91	2.96
10	1.65	4.0	4.49	1.75	1.00	2.71
11	4.11	4.64	5.06	4.45	1.67	1.48
12	3.65	3.20	4.49	3.75	3.00	2.71
13	4.13	3.93	5.06	4.45	3.67	3.48
14	2.82	2.67	3.14	2.38	2.02	2.41
15	2.81	2.42	3.22	2.29	2.09	2.16
16	3.33	3.15	3.78	3.00	2.76	2.93
17	3.09	3.19	4.48	2.94	1.28	2.21
18	3.15	2.88	3.69	2.84	1.50	1.97
19	3.20	2.63	3.77	2.75	1.57	1.72
20	3.66	4.33	3.46	3.46	2.24	2.49
21	3.63	3.41	5.03	3.40	1.80	1.78
22	3.95	3.93	4.90	4.19	3.45	3.41
23	4.68	4.48	6.25	4.75	2.23	3.21

ly. For example, values of logP obtained for compounds **13** were 4.13, 9.93, 5.06, 4.45, 3.67 and 3.48, respectively.

The multivariate comparison of the experimentally obtained values and the coefficients calculated by the computational methods was made by PCA. PCA searches for a few uncorrelated linear combinations of the original variables that capture most of the information in the original variables. The graphical interpretation of the multidimensional space of the data set used by the PCA transformation is obtained with a smaller number of new dimensions of space. The number of principal components (PC) was selected using the criterion of Kaiser. Considering the above eigenvalues and proportions of explained variance it would be reasonable to investigate first two components, which explained more than 78.89% of the variability of the data set. The PC1 is determined by *A*logPs, milogP, logP$_{KOWIN,}$ *X*logP2 and logP$_{Pallas}$, when PC2 is determined by XlogP$_{CAChe}$ and logP$_{Pallas}$. 2D-dimensional projection (Fig. 2) shows that all of original variables are strongly associated with their constituents and negatively correlated with PC1. Moreover, variables logk_0 is not well explained by PC1 and PC2.

The next part of our study was to establish the nature of relationship between selected ADME parameters and log k_0. The absorption process was characterized by protein bound (PB%) and passive absorption (PA%) parameters. The lowest values of PB% were calculated for compounds **5** and **17** (59.24%, 58.58%, respectively) with THIQ moiety and were similar to theophylline. For the rest of compounds PB% was much higher, from approximately 60 to 95 percent. For all compounds the percent of passive absorption is very high and has the same value (100%). For determinant of brain barrier penetration the values of automatically calculated parameters of rate of brain penetration (LogPS) and extent of brain penetration (LogBB) were taken. For all compounds values of LogPS were in the range of -2.2 to -1.3 and were higher than for theophylline (-

2.7). Moreover, contrary to theophylline, for all compounds the LogBB parameter is positive (Table 4).

Results of simulations for compounds **1**-**23** suggest the possibility of good their brain - plasma penetration. The PCA analysis showed that the two principal components (PC1, PC2) explain 90.79% of the total variance (Fig. 3). The PC1 is determined by logk_0, PB%, LogP, logPS and those variables had been strongly interlinked and constituted a homogeneous group. The second component represents the only variable LogBB. All variables are strongly associated with their constituents. The first component is negatively (-0.73; -0.92; -0.95; -0.96) correlated with variables logk_0, PB%, LogP, logPS.

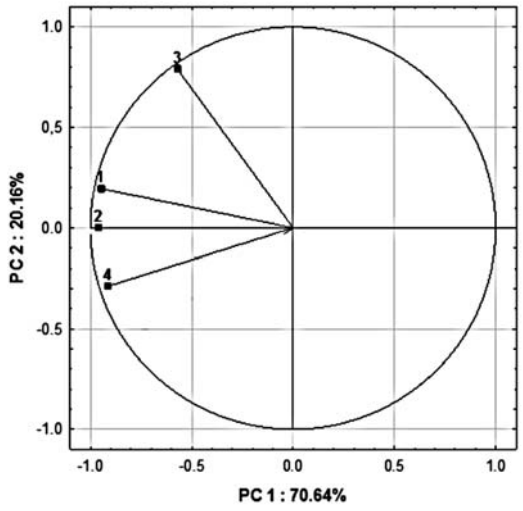


Figure 3. Comparison of the logk_0 and ADME parameters by PCA
1 - logk_0; **2** - PB%; **3** - LogBB; **4** - LogPS

DISCUSSION AND CONCLUSION

Having analyzed the impact of chemical structure on the relative lipophilicity, it was found that it depends on three chemical factors: firstly, the length of alkylene chain linking the nitrogen atom of the tricyclic system with nitrogen atom of the arylpiperazine group; secondly, the presence and type of substituent in the LCAP system or replacement LCAP with THIQ moiety and thirdly, type of substituent at the 7-position of the imidazo[2,1-*f*] theophylline. The analyses revealed that the length of the alkylene chain, connecting the nitrogen atom N8 of the tricyclic secondary amine nitrogen atom, had no significant effect on the value of the relative lipophilicity for the compounds. Logk_0 were in the range of 0.217 to 1.339 for compounds with three methylene unit spacer between imidazo[2,1-*f*] theophylline and arylpiperazinyl moiety. Unexpectedly, for their analogs with four carbon chain, obtained logk_0 values were in the similar range (0.226-0.787). There was no significant effect of the type of substituent in phenyl ring in LCAP fragment on the relative value of lipophilicity but the number of substituents (e.g., comp. **2** *vs*. **3** and comp. **3** *vs*. **4**) and replacement LCAP with THIQ were significant (e.g., comp. **1** vs. **5**). Derivatives of THIQ demonstrated the lowest values of logk_0 (comp. **5**, **10**, **17**, **21**) with one exception of compound **23**. The greatest impact on lipophilicity seems to have a substituent at the 7-position of the imidazo[2,1-*f*] theophylline. Compounds **13** and **22** (derivatives of LCAP and THIQ, respectively) with the highest relative lipophilicity, contain phenyl ring at 7-position of imidazo[2,1-f]theophylline. This trend was mani-

Table 4. The selected ADME values.

Comp	LogPS	LogBB	PB %	PA %	log k_0	Comp	LogPS	LogBB	PB %	PA %	log k_0
1	-2.1	0.27	62.70	100	0.485	**13**	-1.3	0.63	95.49	100	1.339
2	-2.2	0.21	64.65	100	0.597	**14**	-2.0	0.41	65.91	100	0.475
3	-1.7	0.50	77.30	100	0.697	**15**	-2.2	0.38	62.89	100	0.513
4	-1.5	0.45	87.85	100	0.876	**16**	-1.7	0.64	77.75	100	0.529
5	-2.1	0.50	59.24	100	0.217	**17**	-2.0	0.72	58.58	100	0.226
6	-2.0	0.36	68.12	100	0.266	**18**	-1.9	0.51	68.15	100	0.716
7	-2.1	0.35	65.24	100	0.271	**19**	-2.0	0.53	65.49	100	0.687
8	-1.6	0.59	80.50	100	0.708	**20**	-1.5	0.85	81.09	100	0.787
9	-1.4	0.67	88.39	100	0.312	**21**	-1.8	0.90	60.82	100	0.492
10	-2.0	0.67	61.30	100	0.370	**22**	-1.5	0.54	93.04	100	0.733
11	-1.5	0.47	91.31	100	0.715	**23**	-1.5	0.94	90.28	100	0.707
12	-1.6	0.49	90.21	100	1.140	Theophylline	-2.7	-0.04	60.64	100	0.950

fested strongly in the group of THIQ derivatives, where compound **23** with phenyl ring at 7-position of annelated theophylline demonstrated two times higher logk_0 value than their analogue with methyl group. The most of the available computer algorithms for calculation of logP do not recognize well enough new created system, based on the fusion of an additional ring (imidazole) to an already existing one (theophylline). It can be assumed that the results of the rings junction - cyclic guanidine group - significantly alters the properties of the imidazo[2,1-*f*]theophylline. The significant differences between logk_0 and theoretical values (*A*logPs, milogP, logP_{KOWIN}, *X*logP2 and logP_{Pallas}) were the reasons for seeking statistical methods that allow an appropriate way to present relationships between logk_0 and are obtained by use of computational algorithms. PCA analysis showed that none of the computers algorithm gives high correlation with experimental data. The results of ADME assay were compared to experimental logk_0 values by PCA technique and it has showed a high correlation between logk_0 and brain barrier penetration. Moreover, those studies indicate that for the THIQ derivatives of imidazo[2,1-*f*] theophylline values of logk_0 were lower than for LCAPs analogs. So far, THIQ was treated as isostere, with comparable to 1-arylpiperazine moiety physicochemical properties (12) but deprived of receptor 5-HT_{1A} activity.

Summing up, the present study described a simple method for determination of lipophilicity parameters by RP-HPLC of a model series of tricyclic theophylline derivatives. The results above, confirm the legitimacy of the determination of lipophilicity for the investigated compounds by the use of the experimental method. The RP-HPLC technique can be regarded as the better one for the determination of the lipophilicity of tricyclic theophylline derivatives than the computational procedures.

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Conflict of interest

The authors have no conflicts of interest relevant to the ideas and/or contents of the manuscript.

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