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Evaluation of lipophilicity of selected bioactive molecules by HPLC

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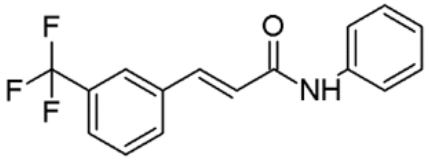
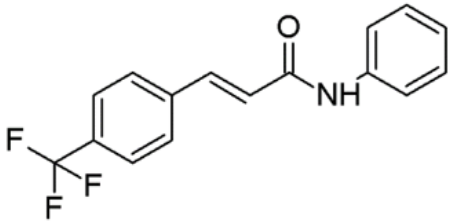
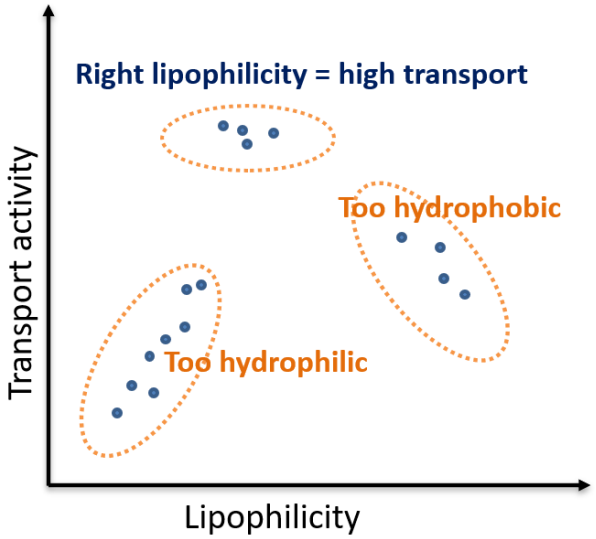
Evaluation of lipophilicity of selected bioactive molecules by HPLC



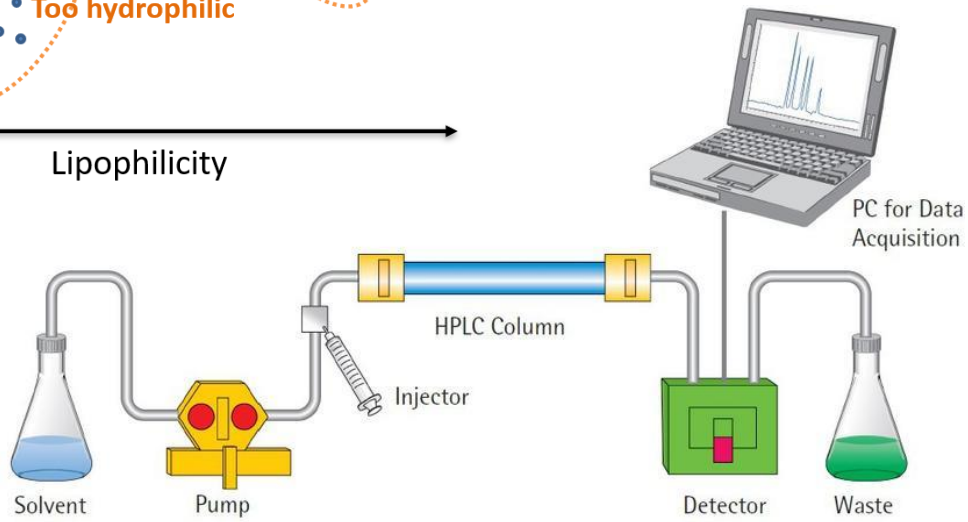
MCF – *meta* derivatives of cinnamic acid



PCF – *para* derivatives of cinnamic acid



Schema of HPLC system



Abstract:

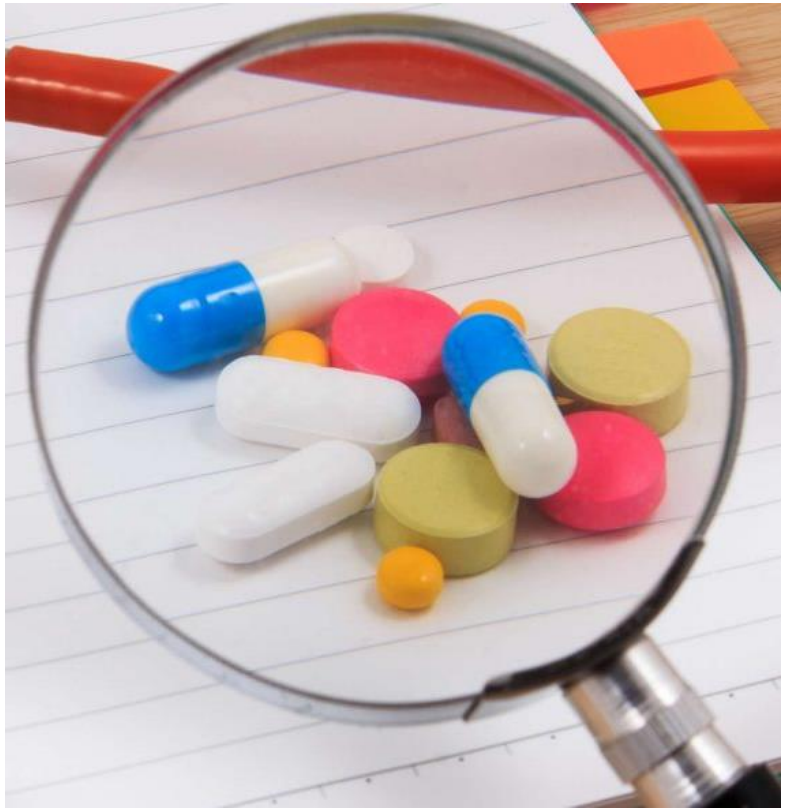
Lipophilicity is one of the important properties of bioactive molecules, according to which the nature of a potential drug is assessed. Evaluation of the lipophilicity of selected cinnamic acid derivatives was performed by high-performance liquid chromatography (HPLC) using reversed (RP) stationary phase C18 and under isocratic conditions. In the case of determining the capacity factor *k*, methanol and water were applied to the system as the mobile phase. The distribution coefficient *D* was determined using a mobile phase composed of methanol and acetate buffer (pH 7.4 or pH 6.5) to ensure a constant pH. This contribution aims to compare the influence of various factors on the lipophilicity of selected trifluoromethyl substituted cinnamanilides, including pH and the position and nature of specific substituents in the anilide portions of the molecules. The results of this study will then be used to evaluate structure-lipophilicity relationships, druglikeness, and structure-activity relationships.

Keywords: *cinnamanilides; HPLC; lipophilicity; log k; log D; log P.*

Lipophilicity

- Research and development of drugs
- Definition of IUPAC [1]
- Key physicochemical property of the active substance [2]

- The affinity of molecule/ group of molecules to the lipophilic environment (in cells) [3]
- Part of ADMET studies [4]
- Lipinski´s Rule of 5 (Ro5) [4] → log $P \leq 5$



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[3] Bunally, S.; Young, R.J. The role and impact of high throughput biomimetic measurements in drug discovery. *ADMET and DMPK* **2018**, *6*, 74-84.

[4] Di, L.; Kerns, E. *Drug-like Properties: Concepts, Structure Design and Methods from ADME to Toxicity Optimization*; Academic Press, 2015.

Mathematical expression of lipophilicity

- **log *P* – Partition coefficient**
  - neutral form of molecules [5]
  - good oral bioavailability of the drug → log *P* = 0–3 [4]

- **log *D* – Distribution coefficient**
  - ionizable substances
  - depends on pH and pK_a values, physiological pH 7.4 [6]

- **log *k* – Retention factor** [4]
  - HPLC
  - is equal to the ratio of retention time of the analyte on the column to the retention time of a non-retained compound

$$k = \frac{t_R^{'}}{t_M}$$

[4] Di, L.; Kerns, E. *Drug-like Properties: Concepts, Structure Design and Methods from ADME to Toxicity Optimization*; Academic Press, 2015.

[5] Wang, T.; Wu, M.-B.; Lin, J.-P.; Yang, L.-R. Quantitative structure–activity relationship: promising advances in drug discovery platforms. *Expert Opinion on Drug Discovery* **2015**, *10*, 1283-1300.

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Methods for the determination of lipophilicity

- **Calculation methods**

Principle of structure, topology or electrotopology

Substructural approaches: *Fragmental* – ChemDraw, ACD/Percepta

Methods of contribution of atoms – Molinspiration

Whole molecule approaches: based on molecular properties and topological descriptors, log *P* - function of molecular properties

- **Experimental methods**

Direct:

shaking-flask method (SFM)[6], slow stirring methods (SSM)[7], potentiometric titration

Indirect:

possible automation, faster analysis, less sample consumption

Chromatographic methods: HPLC, RP-HPLC, TLC, RP-TLC

Electromigration methods: MEEKC, CE

[6] Andrés, A.; Rosés, M.; Ràfols, C.; Bosch, E.; Espinosa, S.; Segarra, V.; Huerta, J.M. Setup and validation of shake-flask procedures for the determination of partition coefficients (logD) from low drug amounts. *European Journal of Pharmaceutical Sciences* **2015**, *76*, 181-191.

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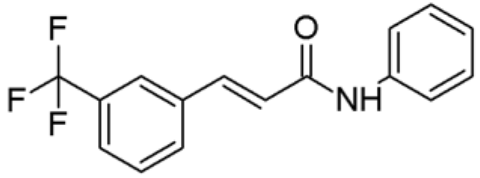
Analytes

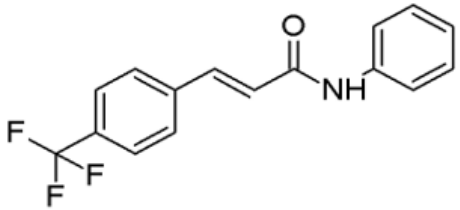
- Two series of 17 samples in position $C_{(3)}$ (*meta* series, MCF) and $C_{(4)}$ (*para* series, PCF) trifluoromethyl substituted cinnamonic acid anilides

- log *k* and log *D* lipophilicity values at pH 6,5 and 7,4

- Factors affecting lipophilicity



Compound	R	Compound	R	Compound	R
MCF 1	H	**MCF 16**	4-Cl	**MCF 57**	2,4-Cl
MCF 11	2-F	**MCF 20**	2-CF_3	**MCF 26**	2,5-Cl
MCF 12	3-F	**MCF 21**	3-CF_3	**MCF 29**	3,5-Cl
MCF 13	4-F	**MCF 22**	4-CF_3	**MCF 33**	3,5-CF_3
MCF 14	2-Cl	**MCF 47**	2,4-F	**MCF 122**	2-Br-4-OCF_3
MCF 15	3-Cl	**MCF 31**	3,5-F		



Compound	R	Compound	R	Compound	R
PCF 1	H	**PCF 16**	4-Cl	**PCF 57**	2,4-Cl
PCF 11	2-F	**PCF 20**	2-CF_3	**PCF 26**	2,5-Cl
PCF 12	3-F	**PCF 21**	3-CF_3	**PCF 29**	3,5-Cl
PCF 13	4-F	**PCF 22**	4-CF_3	**PCF 33**	3,5-CF_3
PCF 14	2-Cl	**PCF 47**	2,4-F	**PCF 122**	2-Br-4-OCF_3
PCF 15	3-Cl	**PCF 31**	3,5-F		

RP-HPLC analysis

High performance liquid chromatograph Waters Alliance 2695 XE:

HPLC system	Waters® 2695 Separation Module S/N (Waters Corp. Milford, MA, USA) F04SM4814M
Detector	Waters® 2487 Dual Wavelength Absorbance Detector S/N J05487402M (Waters Corp.)
Software	Empower 3 Software, chromatography data system (CDS) (Waters Corp.)

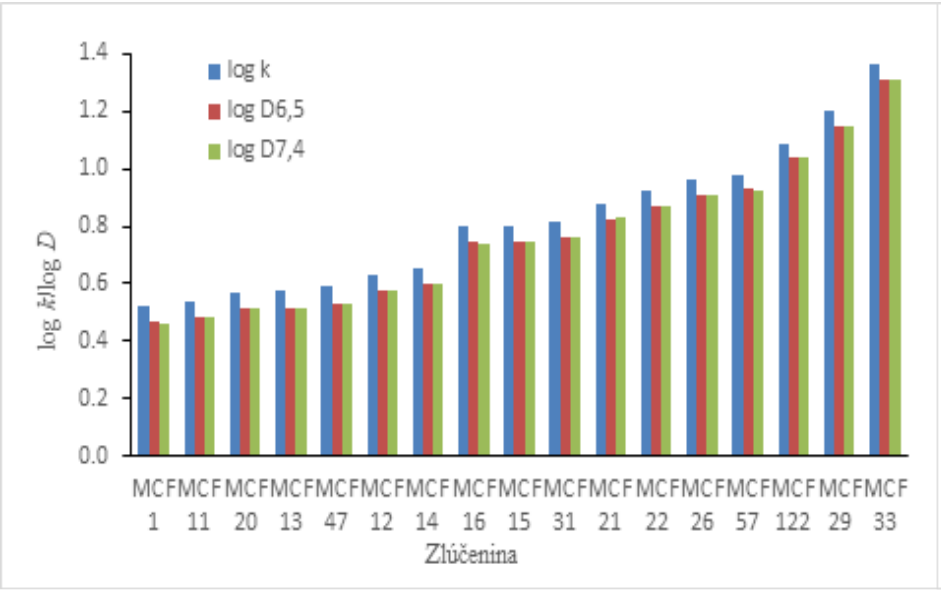
Chromatographic column: Symmetry® C18, 250 × 4,6 mm, 5 µm (Waters)

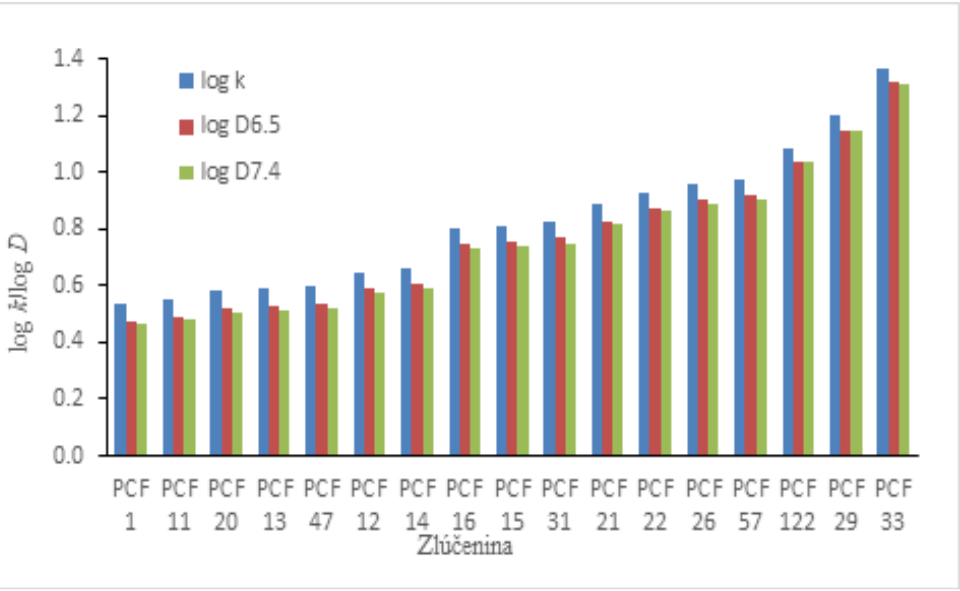
Chromatographic conditions for HPLC analysis

Parameter	Value/description
Stationary phase	C18
Column temperature	40 °C
Mobile phase	A - Methanol: B – water (72:28 v/v) A - Methanol: B – NH_4OAc buffer, pH 7.4 (72:28 v/v) A - Methanol: B – NH_4OAc buffer, pH 6.5 (72:28 v/v)
Type of elution	Isocratic
Flow	1.0 ml/min
Injection	10 µl
Detection	UV detection, $\lambda = 254$ nm

Results and discussion

RP-HPLC analysis: Graphical representation of log *k* and log *D* values obtained using HPLC for MCF (left) and PCF series (right):





log *k* generally exceeded log *D* values, implying that pH actually affects the lipophilicity of the samples

Comparison of lipophilicity of individual compounds

- The most lipophilic compounds: **MCF 33** (R = 3,5-CF_3), resp. **PCF 33** (R = 3,5-CF_3)
- The second most lipophilic compounds: **MCF 29** (R = 3,5-Cl), resp. **PCF 29** (R = 3,5-Cl)
- The least lipophilic compounds: **MCF 1** (R = H), resp. **PCF 1** (R = H)

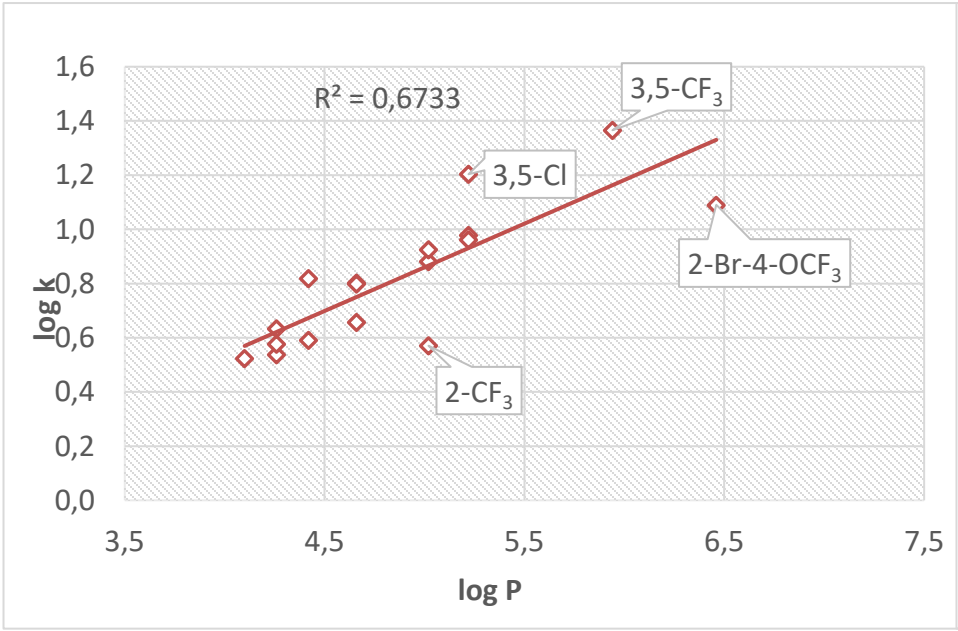
- Factors affecting lipophilicity: **pH**, **nature** and **position of the substituent**
- The highest lipophilicity = *meta* and *para* position

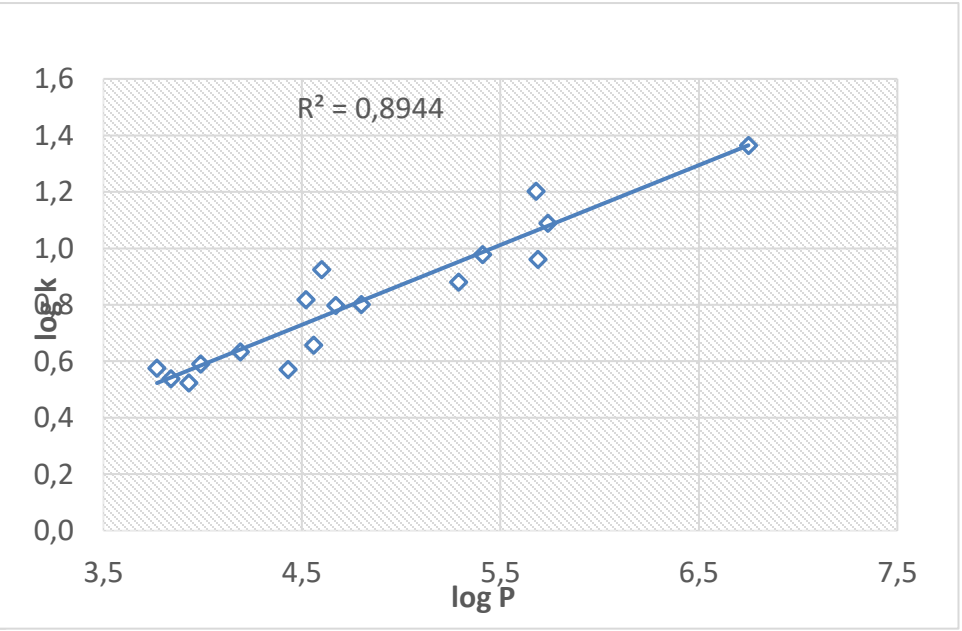
  - for monosubstituted derivatives lipophilicity always increased in order 2 < 4 < 3,
  - for disubstituted derivatives lipophilicity always increased in order 2,5 < 2,4 < 3,5
  - just in case of monoderivatives which were substituted by CF_3 group the order was different: 2 < 3 < 4

- the mathematical conformity of experimentally obtained parameters log *k* and log *D* was evaluated and for all dependencies it was 100%

Comparison of experimental values of lipophilicity with software-predicted values of lipophilicity

Chem Draw Ultra 12.0 (log *P*) (left) **ACD/Percepta 2012** (right)





The same log *P* values for positional isomers (ChemDraw)

Comparison of experimental values of lipophilicity with software-predicted values of lipophilicity

	The least lipophilic substance	The most lipophilic substance	The 2nd most lipophilic substance
Exp. values log *k*	**PCF 1 (R=H)**	**PCF 33** (R=3,5-CF_3)	**PCF 29** (R=3,5-Cl)
Log P–ACD/Percepta	**PCF 16 (R=4-F)**	**PCF 33** (R=3,5-CF_3)	**PCF 122** (R=2-Br-4-OCF_3)
Log P-ChemDraw	**PCF 1 (R=H)**	**PCF 122** (R=2-Br-4-OCF_3)	**PCF 33** (R=3,5-CF_3)
Clog P-ChemDraw	**PCF 11 (R=2-F)**	**PCF 33** (R=3,5-CF_3)	**PCF 29** (R=3,5-Cl)
miLog P-MOLinspiration	**PCF 1 (R=H)**	**PCF 33** (R=3,5-CF_3)	**PCF 122** (R=2-Br-4-OCF_3)

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miLog P-MOLinspiration	**MCF 1 (R=H)**	**MCF 33** (R=3,5-CF_3)	**MCF 122** (R=2-Br-4-OCF_3)

Conclusions

- The lowest lipophilicity shows **MCF/PCF 1** (R = H) and the highest lipophilicity shows **MCF/PCF 33** (R = 3,5-CF_3)
- The **effect of pH** – difference between log *k* and log *D* values
- The most significant influence factor on lipophilicity – **the position of the substitute** - the further the substituents were topologically from the amide bridge (*meta*, *para* position), the higher the lipophilicity
- **ACD/Percepta** was the most suitable software for this analysis
- Other chemical programs are not suitable for predicting lipophilicity of the molecules as they do not distinguish positional isomers

- Experimental data will be provided for further statistical evaluation and for advanced in silico studies
- The data will be used for correlations with biological activities of agents

Acknowledgments

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