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CHROMATOGRAPHIC PARAMETERS IN THE ASSESSMENT OF LIPOPHILICITY AND TOXICITY OF THIOCARBOHYDRAZONE DERIVATIVES

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Abstract: In recent years, the number of studies focused on the design and synthesis of compounds with biological and pharmacological potential has increased significantly. Special attention has been given to molecules with antitumor activity, including thiosemicarbazones and their homologues, thiocarbohydrazones. Since drug development is a complex, lengthy, and expensive process, its optimization in early stages often relies on the application of Quantitative Structure–Activity Relationship (QSAR) approach. By selecting appropriate molecular descriptors, it is possible to quantify the impact of structural modifications on compound's biological activity prior to synthesis, thus reducing the need for extensive experimental work. Lipophilicity as key QSAR descriptor, was determined for thiocarbohydrazones by using a hybrid approach – computationally, through appropriate software tools (logP as a standard measure of lipophilicity), and experimentally, by reversed-phase thin-layer chromatography (chromatographic parameters R_M^0 and m). The results indicated that the nature of the substituent had a greater effect on the chromatographic behavior of thiocarbohydrazones than the applied organic modifier. The correlation between chromatographically and computationally determined lipophilicity values, as well as the acute toxicity parameters (EC_{50}), was assessed by linear regression analysis. The obtained models showed satisfactory predictive performance.

Keywords: thiocarbohydrazones, chromatography, lipophilicity, toxicity.

1. INTRODUCTION

According to the current literature, although organic compounds constitute more than 99% of approved therapeutic agents, increasing attention has been directed toward the pharmacological potential of metal-based drugs [1]. In this context, ligand systems containing multiple donor atoms (N и S), such as thiocarbohydrazones, are of significant interest due to their versatile coordination behavior, redox properties, and ability to modulate biological activity through metal complexation [2]. These compounds have demonstrated a broad spectrum of biological activities, both as free ligands and as metal complexes, including antibacterial, antifungal, antiviral, and antitumor effects [3–5].

Design and development of pharmacologically active compounds is a fundamentally interdisciplinary process that integrates medicinal chemistry, organic synthesis, pharmacology, computational modeling, and molecular biology. Given the complexity, time demands, and high costs associated with drug development, the application of strategies such as structure–activity relationship (SAR), quantitative structure–activity relationship (QSAR), and rational molecular design plays a crucial role in enhancing efficacy, improving selectivity, and reducing toxicity in prospective therapeutic agents.

The establishment of qualitative and quantitative relationships between the chemical structure of a novel molecule and its biological activity relies on the selection and application of appropriate molecular descriptors. These descriptors capture essential physicochemical, electronic, and topological features required for the development of reliable predictive models. Among them, lipophilicity is one of the most important parameters used to predict and interpret the biological activity and pharmacokinetic behavior of compounds. It describes the affinity of a molecule, or a part of a molecule, for a lipophilic environment, that is, its tendency to dissolve in lipids or other nonpolar solvents [6]. Lipophilicity directly influences key pharmacokinetic processes, including absorption and transport across biological membranes, distribution within the body, metabolic stability, elimination, and potential toxicity [7]. The most commonly used parameter to express the lipophilicity of a compound in its non-ionized form is log*P*, defined as the logarithm of the partition coefficient (*P*) describing the distribution of the compound between n-octanol and water [8]. Higher log*P* values correspond to increased lipophilicity. Log*P* values also provide insight into the expected behavior of a compound in a biological environment: lipophilic compounds typically exhibit log*P* > 1, hydrophilic compounds log*P* < -1, and compounds with log*P* = 0 are equally soluble in aqueous and organic phases. In addition, substances with log*P* > 5 are often associated with increased toxicity [9]. When a compound exists in an ionized form, the pH of the solution must be considered, and lipophilicity is more accurately described by the log*D* parameter [10].

In addition to direct determination of logP values, lipophilicity can also be estimated indirectly using chromatographic methods, such as thin-layer chromatography (TLC), expressed by the R_M^0 parameter, and high-performance liquid chromatography (HPLC), expressed by the logk_w parameter [11,12]. Both mentioned parameters represent the retention of a compound when φ = 0 (the retention in a pure water as a mobile phase).

In TLC, R_f values are first calculated based on the distances traveled by the analyte and the solvent front, after which R_M values are determined [13]:

$$R_f = \frac{l_r}{l_f} \quad (1)$$

Extrapolation of the calculated R_M values to zero organic modifier content affords two key chromatographic parameters, as defined by the following equation [14]:

$$R_M = R_M^0 + m\varphi \quad (2)$$

The intercept R_M^0 represents the chromatographic retention constant and is widely used as an alternative descriptor of compound lipophilicity [15–17]. The slope *m*, is a chromatographic parameter related to the specific hydrophobic surface area of the molecule, reflecting the dependence of analyte solubility—and consequently chromatographic retention—on the mobile phase composition. Recent studies have also highlighted the potential of the *m* parameter as an alternative indicator of lipophilicity [18,19].

Given that the design of new ligands primarily requires evaluation of the hydrophobic–hydrophilic balance to enhance cell permeability, as well as assessment of the influence of different substituents on redox properties and metal-binding affinity, a new series of thiocarbohydrazone derivatives was investigated in this study. The effects of substituent nature and organic modifier composition on the chromatographic behavior of the examined thiocarbohydrazones were systematically evaluated. In addition, their lipophilicity was determined experimentally using reversed-phase thin-layer chromatography with two different organic modifiers and computationally using appropriate software tools. The relationships between chromatographic parameters, computationally derived partition coefficient values, and selected acute toxicity parameters were also analyzed. As a result, statistically significant and reliable predictive models were established.

2. EXPERIMENTAL

2.1. Chromatographic measurements

The structures of the investigated thiocarbohydrazone derivatives are presented in Table 1, and their synthesis and characterization have been reported previously [20]. Ethanolic solutions of the compounds (2 mg·cm^{-3}) were applied to commercial reversed-phase TLC plates (RP-TLC C18/UV254s;

Macherey–Nagel, Germany). Chromatograms were developed using a one-dimensional ascending technique with binary mixtures of water and ethanol (J.T. Baker, Deventer, The Netherlands) or dioxane (Sigma-Aldrich, France), without prior saturation of the chromatographic chamber with organic vapor. The volume fraction of the organic modifier (φ) was varied from 0.36 to 0.52. After development, the plates were air-dried at room temperature, and analytes were visualized under UV light at λ = 254 nm as dark spots on a fluorescent background.

For each compound and mobile phase composition, three chromatograms were prepared. Average R_f values were calculated from the distances traveled by the analyte and the solvent front, and subsequently used to determine the chromatographic parameters R_M^0 and m.

2.2. *In silico* determinations

To calculate the values of partition coefficient and toxicity parameters, the following online programs were used: Chemdraw Ultra 12.0, Molinspiration, Molsoft, PreADMET, and SwissADME [21-25]. The Origin 8.1 computer program was used to process the experimental results.

3. RESULTS AND DISCUSSION

3.1. Determination of lipophilicity of thiocarbohydrazone derivatives

The lipophilicity of the investigated thiocarbohydrazone derivatives was initially determined experimentally using reversed-phase thin-layer chromatography in two solvent systems: water–ethanol and water–dioxane. The resulting chromatographic parameters, R_M^0 and m, are presented in Table 2. High values of the regression coefficient r, indicate that the linear relationship between R_M and the organic modifier fraction φ, is valid within the selected experimental range.

The chromatographic retention constant R_M^0, which describes the behavior of a compound in pure water (φ = 0), theoretically depends only on the chemical structure and not on the type of organic modifier. Therefore, identical R_M^0 values were expected for the

Table 1. *Structures of thiocarbohydrazone Derivatives*

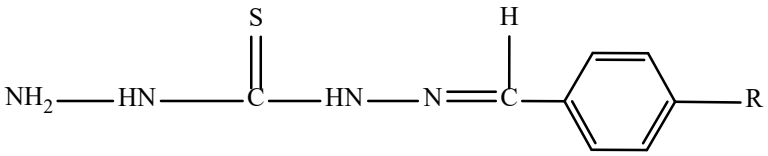
R	
1.H	
2. *p*-CH_3	
3. *p*-F	
4. *p*-Cl	
5. *p*-Br	
6. *p*-NO_2	
7. *p*-OCH_3	
8. *p*-OH	

Table 2. *Values of chromatographic parameters of the tested thiocarbohydrazones*

R	ethanol			dioxane		
	R_M^0	*m*	r	R_M^0	*m*	r
1.H	0.363	-0.841	0.992	0.762	-1.872	0.999
2. *p*-CH_3	0.675	-1.030	0.993	0.973	-2.016	0.999
3. *p*-F	0.471	-0.900	0.999	0.852	-1.940	0.997
4. *p*-Cl	0.763	-1.109	0.996	1.107	-2.118	0.999
5. *p*-Br	0.885	-1.214	0.998	1.216	-2.208	0.997
6. *p*-NO_2	0.382	-0.862	0.998	0.840	-1.924	0.999
7. *p*-OCH_3	0.285	-0.750	0.997	0.681	-1.820	0.999
8. *p*-OH	0.180	-0.676	0.999	0.607	-1.758	0.999

same compound across different modifiers. However, as shown in Table 2, R_M^0 values exhibit slight variations between the applied modifiers, with generally higher values observed in the nonpolar, aprotic dioxane system. As anticipated, R_M^0 is strongly influenced by the nature of substituents on the benzene ring. Within the same organic modifier, derivatives bearing alkyl or halogen substituents display higher R_M^0 values than the unsubstituted parent compound, whereas derivatives with polar substituents show lower values. Among halogenated derivatives, R_M^0 increases in the order –F < –Cl < –Br. Across both modifiers, the highest R_M^0 was observed for derivative with –Br as substitutent, while the lowest corresponded to the most polar –OH derivative.

Additionally, it is evident that the slope values m, vary in parallel with R_M^0 for all tested derivatives in both modifiers. Assuming that the chromatographic parameters R_M^0 and *m* depend on the same physicochemical properties, they are correlated with each other using the linear regression method. The validity of the established R_M^0 – *m* relationships is supported by the high regression coefficients, confirming that these chromatographic parameters reflect the same underlying physicochemical properties (Table 3).

The lipophilicity of the studied thiocarbohydrazones was also evaluated computationally. Table 4 presents the log*P* values predicted by software, serving as standard descriptors of the lipophilicity of the investigated derivatives.

The data in Table 4 indicate that the calculated log*P* values for the same compound vary depending on the mathematical approach used (atomic, fragmentation-based, or whole-molecule methods). As expected, derivatives with nonpolar substituents exhibit higher log*P* values compared to those with polar substituents. Regardless of the mathematical approach, the highest log*P* values were obtained for the derivative with –Br as a substituent, and on average, the lowest for the derivative with –OH group. The obtained results are in accordance with the results obtained experimentally.

3.2. Correlation of experimentally and software determined lipophilicity parameters of studied thiocarbohydrazone derivatives

To assess the applicability of the chromatographic parameters R_M^0 and *m* as alternative measures of lipophilicity, their correlation with computationally determined logP values was analyzed using linear regression (Table 5).

Chromatographic parameters obtained in both applied modifiers showed the best agreement with logP_{cd} values, and the weakest with Wlog*P*. The high regression coefficients confirm the reliability of reversed-phase thin-layer chromatography for assessing the lipophilicity of the selected thiocarbohydrazone derivatives.

Table 3. *R_M^0– m equation for studied derivatives in used modifiers*

modifier	equation	r	sd	p
ethanol	R_M^0 = -0.749 -1.354*m*	0.996	0.024	$< 1 \cdot 10^{-4}$
dioxane	R_M^0 = -1.819 -1.379*m*	0.999	0.008	$< 1 \cdot 10^{-4}$

Table 4. *Software-derived logP values of the studied thiocarbohydrazone derivatives*

R	logP_{cd}	Clog*P*	milog*P*	Mollog*P*	Mlog*P*	Wlog*P*
1. H	1.67	1.54	0.81	1.10	1.07	0.36
2. *p*-CH_3	2.16	2.04	1.26	1.54	1.38	0.67
3. *p*-F	1.83	1.67	0.97	1.16	1.49	0.92
4. *p*-Cl	2.23	2.26	1.49	1.69	1.65	1.01
5. *p*-Br	2.50	2.41	1.62	1.93	1.80	1.12
6. *p*-NO_2	0.83	1.29	0.77	1.03	0.10	0.27
7. *p*-OCH_3	1.55	1.46	0.86	1.05	0.82	0.37
8. *p*-OH	1.28	0.88	0.33	0.53	0.50	0.06

Table 5. *Correlation matrix of R_M^0– logP and m– logP dependencies*

log*P*	r			
	ethanol		dioxane	
	R_M^0	*m*	R_M^0	*m*
logP_{cd}*	0.995	0.995	0.988	0.986
Clog*P*	0.966	0.956	0.936	0.939
milog*P*	0.971	0.964	0.953	0.956
Mollog*P*	0.975	0.973	0.960	0.962
Mlog*P**	0.935	0.944	0.940	0.942
Wlog*P*	0.897	0.889	0.883	0.891

* derivative 6 excluded from correlation

3.3. Correlation of chromatographic parameters with acute toxicity parameters

In order to make a preliminary assessment of the ecotoxicity of the studied thiocarbohydrazone derivatives, the effective concentration EC_{50} values in mg kg^{-1} for selected test organisms were calculated by software (Table 6).

It is evident from Table 6 that the most lipophilic derivative (–Br as a substituent) has the highest toxicity for all test organisms. Also, all tested derivatives show the highest toxicity for the test organism *Minnow*, while they are the least toxic for the organism *Daphnia*.

Table 6. *Software-calculated EC_{50} values of thiocarbohydrazone derivatives for selected test organisms*

R	*Algae*	*Daphnia*	*Medaka*	*Minnow*
1. H	0.115	0.290	0.135	0.063
2. *p*-CH_3	0.070	0.190	0.060	0.034
3. *p*-F	0.090	0.250	0.101	0.030
4. *p*-Cl	0.059	0.134	0.033	0.020
5. *p*-Br	0.052	0.109	0.023	0.016
6. *p*-NO_2	0.104	0.208	0.076	0.040
7. *p*-OCH_3	0.078	0.284	0.133	0.070
8. *p*-OH	0.093	0.312	0.165	0.064

Using the linear regression method, the existence of a dependence between experimentally determined lipophilicity parameters and software-derived EC_{50} values was examined for the studied thiocarbohydrazones (Table 7).

Table 7. *Correlation matrix $R_M^0 – EC_{50}$, $m – EC_{50}$ of the obtained linear dependencies*

EC_{50}	r			
	ethanol		dioxane	
	R_M^0	*m*	R_M^0	*m*
**Algae*	0.982	0.967	0.961	0.954
Daphnia	0.947	0.954	0.974	0.971
Medaka	0.944	0.952	0.966	0.961
Minnow	0.894	0.901	0.919	0.915

*derivatives 7 and 8 are excluded from the correlation

The obtained results indicate the possibility of applying the chromatographic parameters R_M^0 and *m* of the tested thiocarbohydrazone derivatives in the assessment of their acute toxicity.

4. CONCLUSION

Thiocarbohydrazones are important ligands in medicinal chemistry, because they exhibit diverse biological activity both independently and as complexes with metals, and are particularly effective as antitumor agents. Given the fact that the lipophilicity of the compound determines cell permeability, and the nature of the substituent present determines the redox properties and metal binding affinity, the newly synthesized series of thiocarbohydrazones was subjected to the study of their lipophilicity - reverse-phase thin-layer chromatography and computational methods. It was found that the chromatographic behavior of the tested derivatives was affected to a small extent by the organic modifier applied, and to a greater extent by the nature of the substituent present. By applying the linear regression method, a good agreement was established between the chromatographic parameters (R_M^0 and *m*) and the mathematically calculated log*P* values of the tested thiocarbohydrazone derivatives, i.e. their EC_{50} values. The obtained predictive models indicate that chromatographic parameters can be used with satisfactory reliability to describe the lipophilicity and acute toxicity of thiocarbohydrazone derivatives.

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ХРОМАТОГРАФСКИ ПАРАМЕТРИ У ПРОЦЕНИ ЛИПОФИЛНОСТИ И ТОКСИЧНОСТИ ДЕРИВАТА ТИОКАРБОХИДРАЗОНА

Сажетак: Последњих година је забележен значајан пораст броја студија усмерених на дизајн и синтезу једињења са потенцијалном биолошком и фармаколошком применом. Посебна пажња је посвећена молекулима са антитуморским дејством, међу којима се издвајају тиокарбазони и њихови хомолози – тиокарбохидразони. С обзиром да је развој лекова сложен, дуготрајан и финансијски захтеван процес, његова оптимизација у раној фази подразумева примену QSAR (Quantitative Structure–Activity Relationship) приступа. Правилним избором молекулских дескриптора је омогућена квантификација утицаја структурних промена на биолошку активност новог једињења пре његове синтезе, чиме се смањује потреба за опсежним експерименталним испитивањима. Липофилност, као један од кључних дескриптора, је за тиокарбохидразоне одређена хибридни поступком – рачунски, применом одговарајућих софтвера (стандардно мерило липофилности, log*P*) и експерименталним путем, применом танкослојне хроматографије на обрнутим фазама (хроматографски параметри, R_M^0 и m). Испитивања су указала да на хроматографско понашање проучаваних деривата тиокарбохидразона значајнији утицај има природа присутног супституента, него примењени органски модификатор. Усаглашеност између хроматографски и софтверски добијених вредности липофилности, односно вредности параметара акутне токсичности (EC_{50}), је испитана применом методе линеарне регресије, при чему су добијени задовољавајући математички модели.

Кључне речи: тиокарбохидразони, хроматографија, липофилност, токсичност.

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