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Comparision of methods used for estimation of lipophilicity of biologically active phenylthioamides

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Effectiveness of compound actions on living organisms depends on optimal lipophilicity. For studied 2,4-dihydroxyphenylthioamides of microbiocidal properties there were determined the values of lipophilicity parameters: *log* k_w, using high performance liquid chromatography and *log P* – the theoretical distribution coefficient in the n-octanol/water system. The search for a theoretical method was made which could replace chromatographic methods in preliminary choice of compounds of presumably high fungicidal activity.
The obtained results point to the Crippens and Ghose method and the values log P determined in this way correlate well with log kw and allow to choose a lipophilicity range in which the biological activity is the largest.

1. INTRODUCTION

One of essential applications of chromatographic techniques is for determination of hydrophobicity – lipophilicity of organic compounds, mainly those exhibiting biological activity e.g. pesticides, herbicides or fungicides. Hydrophobic character often seems to be the most important physico-chemical parameter in estimation of biological activity changes in a given series of chemical compounds.

This feature plays a decisive role in transfer of the substance through the biological system (cell membrane) and affects also formation of a complex between a compound and a receptor or a biomacromolecule [1].

Only the molecules possessing proper affinity for biological membranes are adsorbed and distributed within an organism and, as a result, they reach the molecular target.

The distribution coefficient of the substance in the water-organic solvent system is the parameter used for determination of hydrophobic-lipophilic character. Of many of them, the n-octanol/water system is the most commonly used for determination of log *P*. It reflects the actual conditions i.e. polar and non-polar phase systems in the biological organisms in the best way [2].

Determination of this parameter using the extraction method based on quantitative determination of concentrations of a given compound in an equilibrium state has numerous limitations which can be eliminated using chromatographic methods [3]. The chromatographic system corresponds better to the biological diffusion and is comparable to the transport of substance within the organism as a dynamic and disorderly process in which molecules interact with biological membranes repeatedly.

Intermolecular interactions between the compound molecules, the receptor or the cell medium are considered in living organisms. Similarly, the substance in the chromatographic system is described through capability of bonding with the eluent and with the filling macromolecules. Intermolecular interactions in HPLC are qualitatively the same type as in biological membranes [4,5].

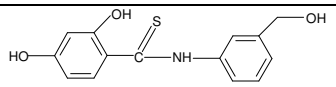
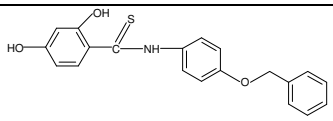
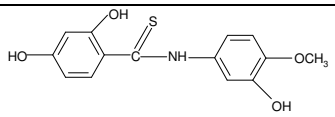
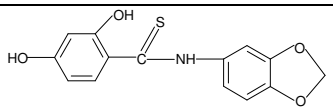
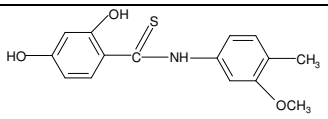
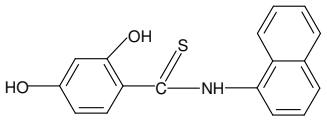
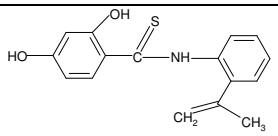
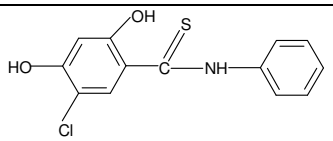
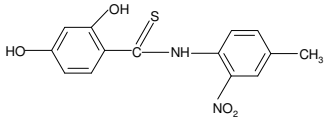
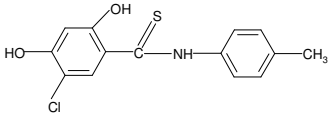
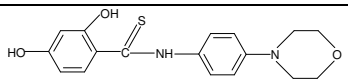
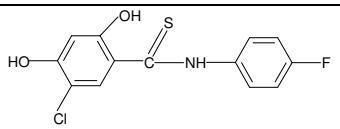
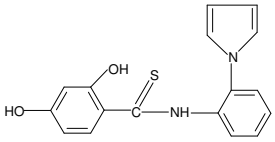
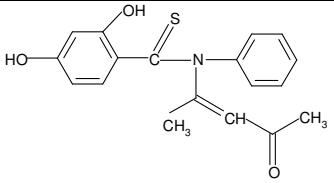
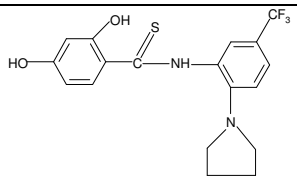
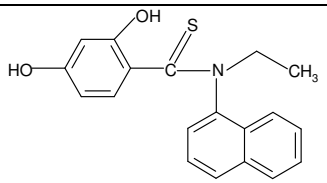
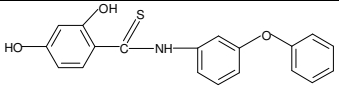
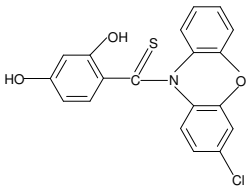
The paper deals with determination of lipophilicity for the structurally modified 2,4-dihydroxyphenylthioamides using the experimental (log k_w-HPLC) and theoretical, log *P* methods as well as relation to compound structure and biological activity for the reference strain *Candida albicans* (10231 ATCC). Experimental determination of log k_w allows for preliminary preselection and suggests applicability of compounds for further investigations. Therefore computer methods of determination of theoretical distribution coefficient log P from the compound structure could facilitate choice and design of biologically active compounds.

Based on the own research, the authors searched for the method of theoretical determination of lipophilicity whose results correlated well with chromatographic methods.

2. EXPERIMENTAL

Synthesis of compounds. The research materials were structurally modified 2,4-dihydroxyphenylthioamides (Table 1) prepared in the Department of Chemistry, University of Nature, Lublin according to the reaction included in the patent claim [6].

Tab. 1. Chemical structure of 2,4-dihydroxyphenylthioamides.

compound	structure	compound	structure
I		X	
II		XI	
III		XII	
IV		XIII	
V		XIV	
VI		XV	
VII		XVI	
VIII		XVII	
IX		XVIII	

Chromatographic investigations – HPLC. The compounds were examined using a chromatograph HPLC Knauer WellChrom (gradient system – 2 pumps + mixer) with the UV detector working at the wave lengths 254 and 320 nm. The column BDS C-18, of the size 150 ×4,6 mm, parking of a 5 μm diameter. The mobile methanol/water phase of the composition 9:1; 8:2; 7:3; 6:4, buffered with the acetate buffer of pH=4.

For each compound and successive mobile phases the volumetric coefficient log k was determined and then based on the linear dependence between log k and the volumetric fraction of organic modifier the value log k_w was determined from the following equation:

$$\log k = \log k_w - S\,\phi \tag{1}$$

where:
S – the constant of the given system
ϕ – the volumetric fraction of organic modifier
k_w – the hydrophobicity index, i.e. the volumetric coefficient obtained for the water as the mobile phase.

Theoretical calculations. The theoretical partition coefficient log P (Table 2) was determined using the computer program CS Chem-Draw Pro ver. 4.5 (Cambridge Soft Corporation), by means of three methods. [7,8,9]

Biological investigations. Studies were carried out according to the document „Reference method for Broth Dilution, Antifungal Susceptibility Testing of Yeast" using the standard strain ATCC 10231 from the collection „American Type Culture of Yeast", University Bird. Manassas.

The sensitivity estimation was made using the plate dilution method. The minimal inhibitory concentration (MIC mg/ml) was determined using the substrate Sabouraud (Bio-Rad). All the same time estimation of reference action of chemotherapeutistics, commonly applied for fighting against fungi (itraconazol and fluconazol) was made.

All compounds were dissolved in 1% DMSO solution and for further dilutions 0.9% NaCl was used. The Sabouraud substrate in the amount of 15 ml was poured over the Petri plates of a 9 cm diameter. After substrate congelation there were compounds of the concentration in the range 200–0.25 mg/l. The studied strain *Candida albicans* 10231 ATCC was inoculated on the Petri plates with the Sabouraud substrate including increasing concentrations of the compound.

The first control system were inoculations of the strain under investigation on the substrate not including the compound, the other control system are the substrates containing 1% DMSO solution. All results were read after 24-hour incubation at 37˚ (Table 3).

Tab. 2. Lipophilicity parameters of compounds obtained by means of the chromatographic method ($\log k_w$), and calculated theoretically ($\log P_1$, $\log P_2$, $\log P_3$)

	$\log k_w$	$\log P_1$	$\log P_2$	$\log P_3$
I	2.49	2.33	2.92	1.55
II	2.52	2.39	2.91	2.04
III	3.36	3.27	3.66	2.85
IV	4.55	4.27	4.77	3.80
V	3.23	3.43	3.83	*
VI	2.49	2.79	3.27	1.97
VII	3.59	3.29	3.46	*
VIII	4.48	4.84	5.32	3.46
IX	4.69	4.44	4.88	4.37
X	4.73	4.51	4.97	3.79
XI	2.96	2.68	3.13	1.53
XII	3.3	3.9	4.45	3.42
XIII	3.29	3.46	3.97	2.92
XIV	3.82	3.95	4.44	3.33
XV	3.94	3.62	4.11	3.06
XVI	3.59	4.08	4.20	3.94
XVII	5.09	5.03	5.49	3.52
XVIII	5.30	5.56	5.92	4.61

*the method does not allow for calculation

3. RESULTS AND DISCUSSION

Quantitative structure-activity dependences (QSAR) are largely connected with the structure as well as physico-chemical and biological properties of ligands ocurring in compounds. A group of 18 derivatives of 2,4-dihydroxyphenylthioamides of different structures was chosen for investigations. As follows from the experimental studies lipophilicity of compounds is significantly differentiated depending on the kind of N-substitution.

Tab. 3. Activity of 2,4-dihydroxyphenylthioamides in relation to the strain *Candida albicans* ATCC 10231 on the substrate Sabouraud in MIC (µg/mL).

comp.	I	II	III	IV	V	VI	VII	VIII	IX
MIC µg/mL	100	100	50	50	100	100	100	200	200
comp.	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII
MIC µg/mL	200	100	50	50	50	50	50	200	100

The values log $P_{(1)}$ calculated using the Crippen method are compared with logk_w and there was obtained the linear dependence (Figure 1) described by equation (2)

$$\log P_{(1)} = 0.9716 \log k_w + 0.1296 \qquad 2)$$

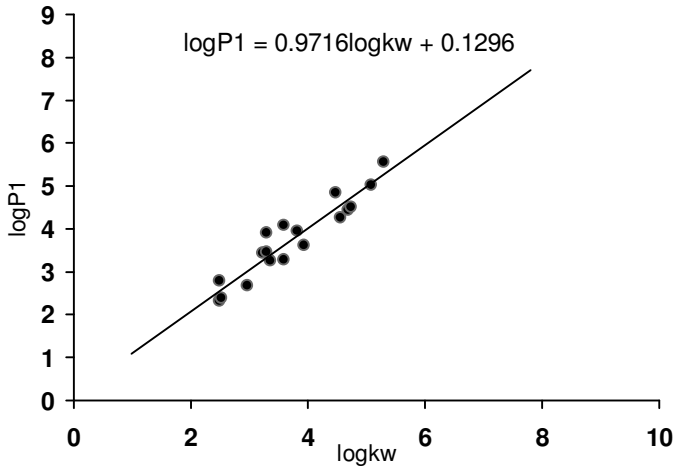


Fig. 1. Dependence between the lipophilicity parametr (log k_w) obtained using the data from HPLC and partition coefficient calculated theoretically (log P_1).

Analyzing the curve it can be stated that the values of the theoretical partition coefficient correspond to lipophilicity of the studied compounds which was

determined by means of the experimental method (HPLC). Though the studied derivatives are differentiated in their structure and molecule composition, deviations were not found for any compounds.
Then the values logk_w as well as log $P_{(2)}$ and log $P_{(3)}$ determined using the respective methods: Viswanadhan and Broto were compared. The obtained linear dependences (Figures 2 and 3) are described using equations 3 and 4.

$$\log P_{(2)} = 0.9415 \log k_w + 0.6848 \quad (3)$$

$$\log P_{(3)} = 0.9178 \log k_w - 0.2823 \quad (4)$$

but in this case correlation is not significant.

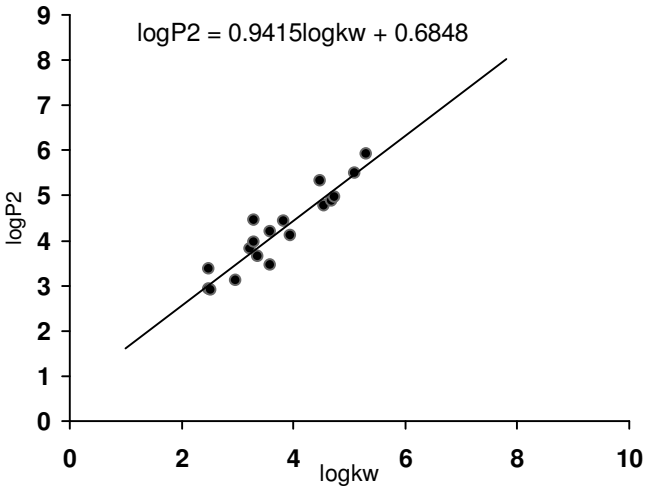


Fig. 2. Dependence between the lipophilicity parametr (log k_w) obtained using the data from HPLC and partition coefficient calculated theoretically (log P_2).

The range of log $P_{(2)}$ values calculated using the Viswanadhan method is slightly larger for all compounds than the theoretical values logk_w , except for compound VII with the pyrrole substituent. However, the Broto method assuming the largest number of various fragmentations for studied atoms gave the weakest dependences, (Figure 3). The values are relatively lower compared to those with HPLC (compound XVI is an exception).

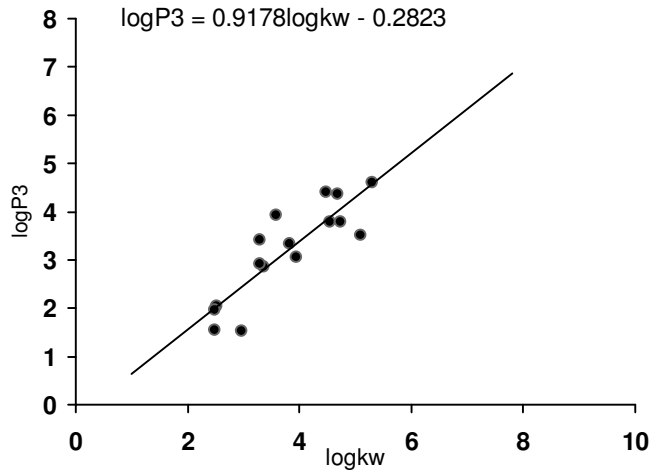


Fig. 3. Dependence between the lipophilicity parametr (log k_w) obtained using the data from HPLC and partition coefficient calculated theoretically (log P_3).

Determination of compound hydrophobicity allows to search for correlation to biological activity. For 2,4-dihydroxyphenylthioamides efficiency of action on the standard strain *C. albicans* ATCC 10231, MIC (minimal concentration inhibiting fungi development) is expressed in the range 50–200µg/ml. As follows from the obtained results, high lipophilicity of compounds towards *C. albicans* is not a factor promoting biological activity. Phase affinity increases probability of molecule bonding in the lipoprotein layer which restricts migration to enzymatic activity sites and capability of reaching a molecular target. For the compounds of logk_w>4, the values MIC are the highest (compound IV is an exception). Relative lipophilicity increase of this derivative may be associated with the presence of 2-isopropenyl substituent affecting the confirmation equilibrium states after incorporation of the group –SH into the cyclic 1,3-benzthiazine system.

Good fungicidal activity MIC 50µg/ml is found in the compounds of average lipophilicity, logk_w 3.29–3.94, [log $P_{(1)}$ 3.27–4.08], whose affinity for cell structures ensures optimal transport inside the cells of *C. albicans* and inhibitory action.

In compounds XIII-XV the presence of chlorine in the position C-5 of phenol ring affects lipophilicity of molecules in a stabilization way, independent of modification orientation of N-aryl system- logk_w 3.29–3.94, [log$P_{(1)}$ 3.46–3.95]. Lipophilicity increase of these compounds in reference to the unsubstituted systems-Cl [10] results in higher biological activity [11].

Good correlation between the parameters describing molecule lipophilicity and its antifungal activity is observed for the studied group of compounds. It is promising that in the future while searching for substances of strong antifungicidal activity, it will be possible to choose systems for synthesis based on lipophilicity and molecule structure which can simplify the search and limit the costs significantly.

Application of log *P* in this estimation will be very important as system hydrophobicity can be characterized based on the theoretical structure. The Crippen method seems to show behaviour of the molecule in the chromatographic system and its presumable interactions in the organism in penetration through the cell structures in the most accurate way.

The results of the paper will be used in further investigations using a larger group of compounds and phytopathogeneous fungi as well as derivatives of acaricidial properties.

4. REFERENCES

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