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Experimental and Calculation Procedures for Molecular Lipophilicity: A Comparative Study for 3,3'-(2-Methoxybenzylidene)bis(4-hydroxycoumarin)*

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Key words
• lipophilicity
• 3,3'-(2-methoxybenzylidene)-bis(4-hydroxycoumarin)
• MBbisHC
• HIV-integrase

In our work, the log *P* value for 3,3'-(2-methoxybenzylidene)bis(4-hydroxycoumarin) (MBbisHC) was experimentally determined (»shake-flask« method) and calculated using six different computer programs: HyperChem 7.0 (based on atom contributions), XLOGP (based on atom contributions), KowWin (based on atom/fragment contributions), CLOGP (based on fragmental contributions), ALOGPS 2.1 (based on atom-type electrotopological-state indices and neural network modeling), and IA logP (based on atom-type electrotopological-state indices and neural network modeling). The experimental and calculated log *P* values were correlated. The best result was achieved using the HyperChem 7.0 program, which resulted in a nearly perfect agreement between the experimentally observed and calculated log *P* values.

INTRODUCTION

The HIV integrase, the only enzyme required for HIV integration, is an attractive target for selective anti-HIV therapy since there is no other known functional counterpart in human cells.[1] Recently, reports have appeared on high HIV integrase inhibitory potency of certain coumarin dimers' analogues. In previous studies it was shown that the active pharmacophore consisted of a coumarin dimer containing an aryl substituent on the central linker methylene. For several dimers, lipophilicity (logarithm of n-octanol/water partition coefficient – log *P*) was considered a useful parameter in bioactivity. A general trend was observed in which increased lipophilicity (log $P \geq 2.5$) of the central linker substituent was associated with increased anti-HIV potency ($IC_{50} \leq 20$).[2] Lipophilicity can be determined by experimental methods (»shake-flask« and potentiometric methods). The widespread application of lipophilicity to drug design explains the need for quick procedures to quantify molecular lipophilicity, particularly at the screening level.[3,4]

Log *P* value for 3,3'-(2-methoxybenzylidene)bis(4-hydroxycoumarin) (MBbisHC) was experimentally determined (»shake-flask« method) and calculated using six computer programs: HyperChem 7.0 (based on atom contributions),[5] XLOGP (based on atom contributions),[6] KowWin (based on atom/fragment contributions),[7] CLOGP (based on fragmental contributions),[8] ALOGPS

* Dedicated to Professor Nenad Trinajstić on the occasion of his 65th birthday.
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2.1 (based on atom-type electrotopological-state indices and neural network modeling),[9–12] and IA logP (based on atom-type electrotopological-state indices and neural network modeling).[13]

EXPERIMENTAL

»Shake-flask« Method

»Shake-flask« n-octanol–phosphate buffer$_{(0.15\ M,\ pH = 7.4)}$ partition coefficient for the target compound was determined at room temperature. Buffer and n-octanol (Sigma) were saturated with each other. The compound, MBbisHC, (Figure 1) was dissolved in dimethylsulfoxide (p.a. Kemika) and ethanol (p.a. 96 %, Kemika) at a concentration of 1.22 mg mL^{-1} to give the stock solution.

The amounts of sample were chosen so that absorption of 0.2 to 0.8 was reached after partitioning. The experiments were performed in the system n-octanol : phosphate buffer = 1 : 75 (vol.). All solutions were pipetted into glass vials, n-octanol and stock solution were added with a microliter syringe. The phases were shaken together on a mechanical shaker (Viggo, HAL SINGBORG) for 30 minutes, centrifuged (Hettich ROTOFIX 32) at 2500 rpm for 20 min to afford complete phase separation, and the n-octanol phase was removed. Absorbance of the buffer phase was measured spectrophotometrically using a Shimadzu UV-1601 spectrophotometer at 204 nm.[14]

P values were calculated using Eq. 1.

$$P = \frac{y - x}{x} \cdot \frac{V_{(\text{buffer})}}{V_{(\text{n-octanol})}} \qquad (1)$$

P – partition coefficient; y – total amount of MBbisHC; x – amount of MBbisHC in the buffer phase after partitioning; $V_{(\text{buffer})}$ – volume of phosphate buffer; $V_{(\text{n-octanol})}$ – volume of n-octanol.

The results obtained in experiments are summarized in Table I.

Statistical analysis of experimental data was performed using the statistical program StatSoft 6.0. The experimentally determined log P values show good correlation (r = 0.9277) between experimental data. The mean value of all experimental log P values is 2.4967 (Figure 2).

Calculation of Lipophilicity (log P*)*

A number of different computer programs for the calculation (prediction) of lipophilicity of chemical compounds, based on their structure, have been recently developed. In our work, six computer programs based on different calculation methods for computing log P have been compared.

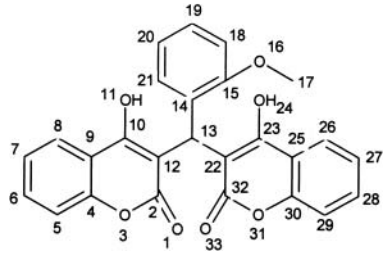

Figure 1.
3,3'-(2-Methoxybenzylidene)bis(4-hydroxycoumarin) (MBbisHC).

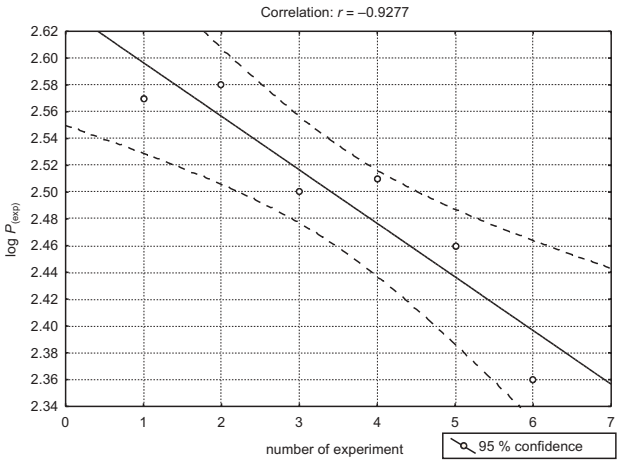

Figure 2. Correlation of the experimentally determined log P values.

TABLE I. Results of experimental determination of log P value by the »shake-flask« method for MBbisHC

c_1	V	A	$\bar{A}$	c_2	P	log P
6×10^{-4}	26.4	0.248	0.247	0.135	370.78	2.57
		0.244				
		0.251				
		0.245				
7×10^{-4}	30.8	0.205	0.225	0.155	379.77	2.58
		0.223				
		0.225				
		0.245				
1×10^{-3}	44.0	0.321	0.329	0.255	317.96	2.50
		0.362				
		0.307				
		0.328				
1.5×10^{-3}	66.0	0.444	0.449	0.377	323.71	2.51
		0.442				
		0.452				
		0.461				
2×10^{-3}	88.0	0.623	0.632	0.554	286.7	2.46
		0.645				
		0.624				
		0.637				
2.5×10^{-3}	110.0	0.721	0.741	0.821	230.13	2.36
		0.688				
		0.774				
		0.781				

c_1 – concentration (mol L^{-1}) of stock solution in n-octanol before partitioning; V – volume (μL) of stock solution; A – absorbance in buffer solution after partitioning (λ = 204 nm); $\bar{A}$ – arbitrary absorbance in buffer solution after partitioning (λ = 204 nm); c_2 – concentration (mol L^{-1}) in buffer solution after partitioning; P – partition coefficient; log P – logarithm of the partition coefficient.

TABLE II. Experimentally determined and calculated log *P* values by six alternative theoretical methods for MBbisHC

	»shake-flask«	HyperChem 7.0	XLOGP	LogKow	CLOGP	ALOGPS 2.1	IA logP
log *P*	2.5	2.54	4.54	3.64	4.32	3.06	4.11
$\Delta_{(\log P_{est.} - \log P_{exp.})}$	/	0.04	2.04	1.14	1.82	0.56	1.61

The quality of these programs was evaluated by how well the computed log *P* values agreed with the experimentally determined log *P* value for (MBbisHC). In our work, SMILES (**S**implified **M**olecular **I**nput **L**ine **E**ntry **S**ystem) notation created by the structure drawing program CambridgeSoft's ChemDrawPro was used as chemical structure input for all programs, except HyperChem 7.0.[15] No »missing fragments« occurred in any of the proposed methods.

HyperChem 7.0

The computer program HyperChem 7.0 calculates log *P* values according to Ghose, Prichett and Crippen.[5] Their method avoids correction factors and estimates lipophilicity on the individual atom basis. The program lists 51 atom contributions for each atom-type. The log *P* value is estimated by summing up all atom contributions.

XLOGP

The XLOGP is another atom-additive method, based on summation of atomic contributions, but it includes ten additional correction factors for some intramolecular interactions. Atoms are classified by their hybridization states and neighboring atoms (Table II). There was no correction factor for the target compound.

(LogKow) KowWin

The LogKow (KowWin) program calculates log *P* values of organic chemicals using the atom/fragment method. The log *P* value (3.6383) of MBbisHC is calculated by simply summing up all atom/fragment contribution values, correction factors (multi-alcohol correction and cyclic ester-olefinic type correction) and the linear equation constant (0.229).

CLOGP

Although some methodology reversions and extensions have been made during the algorithm development, the CLOGP program is based on the fragmental method developed by Leo and Hansch. The calculation result is accompanied by the picture of chemical structure as generated by the DEPICT algorithm. Aromaticity of coumarin rings is indicated by circles inside aromatic rings. The result of the log *P* value calculation for MBbisHC by the CLOGP program is displayed in the »Map Box«. The first line in the »Map Box« is the SMILES notation of the compound. The second line indicates Isolating Carbons (A – aliphatic, a – aromatic), the third numbers of polar fragments, and the fourth the location of hydrogen atoms. The last lines indicate the location of atoms in the rings. All fragments and a variety of different types of correction factors to account for the way in which different fragments influence one another are listed at the end of the »Map Box«. After fragment constants are assigned to all fundamental fragments, and all corrections are accounted for, then the summation is formed and the estimated log *P* value (4.315) is obtained.

ALOGPS 2.1

The ALOGPS 2.1 package included a program for predicting the lipophilicity of chemical compounds. A method for predicting log *P* values is based on atom-type electrotopological-state (E-state) indices and neural network modeling developed by I. V. Tetko *et al*.[10–12] This method combines electronic and topological characters to predict lipophilicity of the analyzed molecules. The analyzed compound (MBbisHC) was entered using the SMILES notation and the ALOGPS 2.1 program calculated E-state indices and predicted the log *P* value (3.06).

IA logP

The IA logP program predicts lipophilicity of compounds using neural network algorithms and E-state atom indices.

DISCUSSION

In the relation between the structure and anti-HIV activity, coumarin-based inhibitors of HIV integrase lipophilic character log *P* appear to be among the most important physicochemical parameters. The aim of our work is to determinate the lipophilicity of MBbisHC and to correlate it with the log *P* values estimated by six alternative theoretical methods (Table II).

All computer programs show to be relatively simple and applicable to QSAR studies. Though the programs are highly useful for predicting the solvation properties, they cannot easily substitute for experimental methods (»shake-flask« method). There is nearly perfect agreement between the experimentally observed and calculated log *P* (HyperChem7.0) values. But, we must bear in mind that one compound is not statistically sufficient. Due to high lipophilicity of the analyzed compound, anti-HIV activity MBbisHC will be investigated in our further work.

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SAŽETAK

Eksperimentalni i računski postupci određivanja lipofilnosti za 3,3'-(2-metoksibenziliden)bis(4-hidroksikumarin)

Marica Medić-Šarić, Ana Mornar, Tanja Badovinac-Črnjević i Ivona Jasprica

U ovome radu log *P* vrijednost za 3,3'-(2-metoksibenziliden)bis(4-hidroksikumarin) određena je eksperimentalno (»shake-flask« metodom) i računski uporabom šest različitih računalnih programa: HyperChem 7.0, XLOGP, KowWin, CLOGP, ALOGPS 2.1 i IA logP. Najbolji rezultat dobiven je pomoću programa HyperChem 7.0, koji pokazuje najbolje slaganje s eksperimentalno određenim log *P* vrijednostima.