МОЛЕКУЛЯРНА БІОФІЗИКА

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ON THE POSSIBILITY OF THEORETICAL PREDICTION OF BENZANTHRONE DYE LIPOPHILICITY

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The present study was aimed at comparing the prediction power of several computational methods in evaluating the lipophilicity (log*P*) of a series of newly synthesized benzanthrone derivatives, tentatively divided into three groups: 1) aminobenzanthrones, 2) amidinobenzanthrones, and 3) bromine-containing amidinobenzanthrones. Experimental partition coefficients ($\log K_p$) were derived from fluorescence titration experiments. Analysis of the correlation coefficients between the experimental and theoretically predicted lipophilicity values showed that generally $\log K_p$ rather poorly correlates with log*P* of the examined compounds. The best agreement between calculated and experimental lipophilicities was observed for KOWWIN (group1), XLOGP3 (group2) and ALOGPs (group 3) methods, pointing to the importance of different structure- and property-based factors in determining the dye lipophilicity. It is concluded that computed logP, with careful choice of calculation method, can be effectively used for prediction of membrane partition properties of benzanthrones, in combination with other molecular and quantum-chemical descriptors.
KEY WORDS: benzanthrone dyes, lipophilicity, correlation coefficient.

О ВОЗМОЖНОСТИ ТЕОРЕТИЧЕСКОГО ПРЕДСКАЗАНИЯ ЛИПОФИЛЬНОСТИ БЕНЗАНТРОНОВЫХ КРАСИТЕЛЕЙ

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Цель данной работы заключалась в сравнительной оценке возможности теоретической оценки липофильности серии новых производных бензантрона, условно разделенных на три группы, 1) аминобензантроновые, 2) амидинобензантроновые и 3) амидинобензантроновые бром-содержащие соединения. Экспериментальные коэффициенты распределения ($\log K_p$) были определены методом флуоресцентного титрования. Анализ коэффициентов корреляции между между экспериментально определенными коэффициентами распределения и расчетными значениями липофильности (log*P*) показал, что в общем случае $\log K_p$ слабо коррелирует с log*P*.

Обнаружено, что самые высокие коэффициенты корреляции обеспечиваются методом KOWWIN для первой, XLOGP3 для второй и ALOGPs для третьей группы исследуемых красителей, что свидетельствует о влиянии как структурных, так и физико-химических факторов на липофильность бензантронов. Сделан вывод о том, что расчетные значения липофильности, при тщательном выборе метода расчета, могут быть эффективно использованы в предсказании мембранного распределения бензантронов, в комбинации с другими молекулярными и квантово-химическими дескрипторами.
Ключевые слова: бензантроновые зонды, липофильность, коэффициенты корреляции.

ПРО МОЖЛИВІСТЬ ТЕОРЕТИЧНОГО ПЕРЕДБАЧЕННЯ ЛІПОФІЛЬНОСТІ БЕНЗАНТРОНОВИХ БАРВНИКІВ

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Мета даної роботи полягала у порівняльній оцінці можливості теоретичної оцінки ліпофільності серії нових похідних бензантрону, умовно розділених на 1) амінобензантронові, 2) амідінобензантронові та 3) бромвміщуючі амідінобензантронові сполуки. Експериментальні коефіцієнти розподілу ($\log K_p$) були визначені методом флуоресцентного титрування. Аналіз коефіцієнтів кореляції між експериментально отриманими коефіцієнтами розподілу і розрахунковими значеннями ліпофільності (log*P)* показав, що у загальному випадку $\log K_p$ слабко корелює з log*P*. Встановлено, що найвищі коефіцієнти кореляції забезпечуються методом KOWWIN для першої, XLOGP3 для другої і ALOGPs для третьої групи досліджуваних барвників, що свідчить про вплив як структурних, так і фізико-хімічних факторів на ліпофільність бензантронів. Зроблено висновок про те, що розрахункові значення ліпофільності, при ретельному виборі методу розрахунку, можуть ефективно використовуватись для прогнозування мембранного розподілу бензантронів, у комбінації з іншими молекулярними і квантово-хімічними дескрипторами.

Ключові слова: бензантронові зонди, ліпофільність, коефіцієнти кореляції.

During the past decades benzanthrone derivatives attract ever growing interest due to their favorable photophysical properties, such as large extinction coefficients, marked Stokes shift, negligible fluorescence in a aqueous phase, high sensitivity of fluorescence parameters to environmental polarity, etc [1,2]. These unique spectral characteristics resulted in the intensive use of benzanthrones in a wide variety of scientific and technological areas as disperse dyes for textiles, polymers, daylight fluorescence pigments and laser dyes [3,4]. In addition, benzanthrone dyes are successfully recruited as effective microenvironmental sensors for monitoring structural changes of biological macromolecules and their assemblies [5-7]. To exemplify, 3-methoxybenzanthrone was employed as a solvatochromic DNA – intercalating fluorescent dye [5]. Another benzanthrone, ABM was found to surpass classical amyloid marker Thioflavin T in its ability to detect pathological protein aggregates, amyloid fibrils [6]. Furthermore, these dyes were reported to display marked sensitivity to the changes in immune status of a human organism at different pathologies [8,9]. However, application of benzanthrones to the examination of living organisms is complicated by their high toxicity [10]. Epidemiological studies indicate that skin, respiratory, gastrointestinal, nervous and hemopoietic systems are affected during benzanthrone dyes exposure [11]. In this regard, it is important to determine the lipophilicity (log*P*) of these dyes, since in describing the dye properties, such as solubility, absorption, toxic action, plasma protein binding, *etc*. lipophilicity is considered as a principal physicochemical descriptor.

The term “lipophilicity” is traditionally expressed as the logarithm of the partition coefficient of a solute between two essentially immiscible solvent phases (aqueous and non-aqueous, in practice, water and *n*-octanol). It is important to note that log*P* provides characterization of a certain compound or its molecular fragment in the context of its affinity for a lipid environment. Unfortunately, experimental determination of lipophilicity is often cumbersome and time-consuming process, especially for zwitterionic and highly lipophilic or polar compounds [12]. For this reason, a wide variety of computational methods based on different theoretical approaches are currently used to predict lipophilicity of a certain compound.

The aim of the present study was to evaluate different theoretical approaches with respect to their suitability for lipophilicity determination of benzanthrone dyes.

To this end, experimentally determined partition coefficients ($\log K_p$) were compared with computed log*P* values for a series of newly synthesized benzanthrone derivatives (Fig. 1). According to their structure, the examined dyes where divided into three groups: 1) aminobenzanthrones (A6, ABM, A8, P9, P14), 2) amidinobenzanthrones (AM19, AM21, AM20, AM12) and 3) bromine-containing amidinobenzanthrones (AM2(23), AM4(23), AM15(23), AM18(23)).

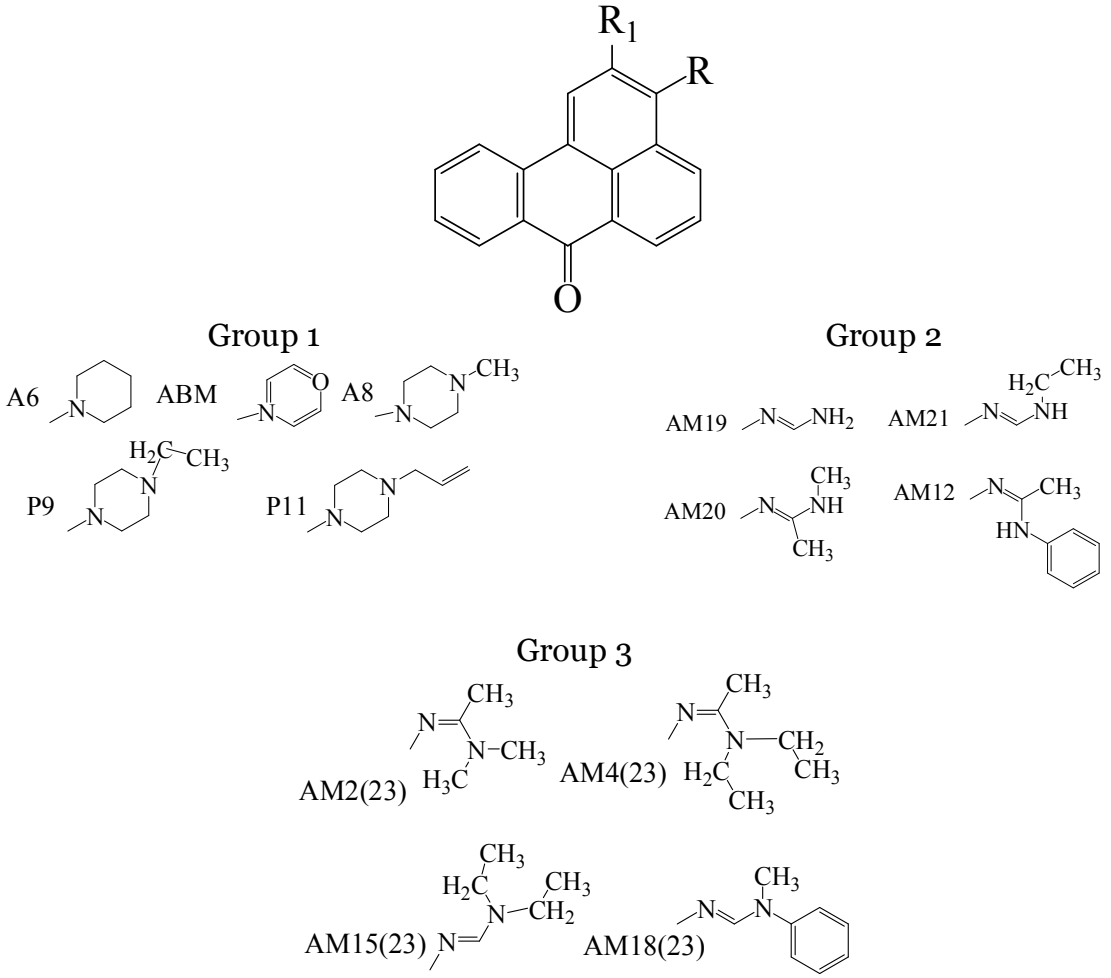

Fig. 1. Chemical structures of benzanthrone derivatives. For group 3 R_1= Br.

MATERIALS AND METHODS

Experimental

Egg yolk phosphatidylcholine were purchased from Biolek (Kharkov, Ukraine). Benzanthrone dyes were synthesized at the Faculty of Natural Sciences and Mathematics of Daugavpils University [13]. All other chemicals were of analytical grade and used without further purification.

Unilamellar lipid vesicles were prepared from PC by the extrusion method [14]. The thin lipid film was obtained by evaporation of lipid ethanol solution and then hydrated with 1.2 ml of 5 mM Na-phosphate buffer (pH 7.4). Lipid suspension was extruded through a 100 nm pore size polycarbonate filter. The dye-liposome mixtures were prepared by adding the proper amounts of the probe stock solutions in ethanol to liposome suspension.

Steady-state fluorescence spectra were recorded with LS-55 spectrofluorimeter (Perkin Elmer, Great Britain) equipped with magnetically stirred, thermostated cuvette holder. Fluorescence measurements were performed at 20°C using 10 mm path-length quartz cuvettes.

Calculation of logP

The lipophilicity parameter (log*P*) was calculated for the above benzanthrones using the online Virtual Computation Chemistry Laboratory (www.vcclub.org), providing the opportunity for interactive log*P* prediction with a variety of algorithms, such as Pharma Algorithm (AC_log*P* parameter), Molinspiration algorithm implemented in the DragonX software (miLogP, KOWWIN, ALOGP and MLOGP parameters) [15,16], XLOGP2 and XLOGP3 programs (XLOGP2 and XLOGP3 parameters) [17] and ALOGPS 2.1 program (ALOGPs method) [18].

THEORY

Total concentration of the dye distributing between aqueous and lipid phases (Z_{tot}) can be represented as:

$$Z_{tot} = Z_F + Z_L \tag{1}$$

where subscripts *F* and *L* denote free and lipid-bound dye, respectively. The coefficient of dye partitioning between the two phases (K_P) is defined as [19]:

$$K_P = \frac{Z_L V_W}{Z_F V_L} \tag{2}$$

here V_W, V_L are the volumes of the aqueous and lipid phases, respectively. Given that under the employed experimental conditions the volume of lipid phase is much less than the total volume of the system V_t, we assume that $V_W \approx V_t = 1$ dm^3. It is easy to show that

$$Z_F = \frac{Z_{tot} V_W}{V_W + K_P V_L} = \frac{Z_{tot}}{1 + K_P V_L} \tag{3}$$

The dye fluorescence intensity measured at a certain lipid concentration can be written as:

$$I = a_f Z_F + a_L Z_L = Z_F \left(a_f + a_L \frac{K_P V_L}{V_W} \right) = Z_F \left(a_f + a_L K_P V_L \right) \tag{4}$$

where a_f, a_L represent molar fluorescence of the dye free in solution and in a lipid environment, respectively. From the Eqs. (3) and (4) one obtains:

$$I = \frac{Z_{tot}(a_f + a_L K_P V_L)}{1 + K_P V_L} \tag{5}$$

The volume of lipid phase can be determined from:

$$V_L = N_A C_L \sum v_i f_i \tag{6}$$

where C_L is the molar lipid concentration, f_i is mole fraction of the *i-th* bilayer constituent, v_i is its molecular volume taken as 1.58 nm^3 for PC [20].

The relationship between K_p and fluorescence intensity increase (ΔI) upon the dye transfer from aqueous to lipid phase can be written as [19]:

$$\Delta I = I_L - I_W = \frac{K_p V_L (I_{\max} - I_W)}{1 + K_p V_L} \tag{7}$$

where I_L is the fluorescence intensity observed in the liposome suspension at a certain lipid concentration C_L, I_W is the dye fluorescence intensity in a buffer, $I_{\max}$ is the limit fluorescence in a lipid environment.

RESULTS AND DISCUSSION

The photophysical properties of benzanthrone dyes are known to be strongly dependent on the electron donor-acceptor interaction within chromophoric system [21]. The occurrence of ICT state is controlled by electron-donating and electron-accepting powers of the fluorophore functional groups. The dyes under study are asymmetrical amino- and amidino-benzanthrone derivatives of orange-red colour. The first step of the study was focused on experimental determination of the dye partition coefficients. Fluorescence spectroscopy technique was employed to quantify the dye partitioning between aqueous and lipid phases. Emission spectra of the dyes were recorded in buffer solution and suspension of PC liposomes.

Table 1. Experimental (log*Kp*) and theoretical (log*P*) lipophilicity values calculated using different theoretical approaches for benzanthrone dyes

Compound	log*Kp*	ALOGPs	AC logP	MilogP	ALOGP	MLOGP	KOWWIN	XLOGP2	XLOGP3
Group 1									
A6	4.48	5.17	5.47	5.79	4.91	4.31	6.27	5.83	5.28
ABM	4.2	3.82	4.26	4.72	3.68	3.26	4.52	4.57	4.06
A8	3.95	3.92	4.38	4.77	3.95	3.48	4.49	4.61	4.24
P9	4.12	4.48	4.81	5.15	4.3	3.69	4.98	5.03	4.61
P14	4.38	5.72	6.09	6.57	5.86	4.69	6.47	6.48	6.20
Group 2									
AM19	4.3	3.24	3.37	4.11	2.93	3.7	3.76	3.67	3.38
AM21	5.17	3.57	4.33	4.86	3.71	4.16	4.71	4.55	4.15
AM20	4.36	3.93	4.17	4.93	3.41	4.16	5.38	4.37	3.77
AM12	5.26	5.52	5.75	6.63	5.26	5.3	7.18	5.93	5.32
Group 3									
AM2(23)	4.42	4.8	5.09	5.92	4.69	4.98	6.48	5.73	4.64
AM4(23)	4.48	5.61	5.96	6.67	5.39	5.41	7.46	6.57	5.37
AM15(23)	4.47	5.33	5.68	6.22	5.34	5.2	6.31	6.34	5.39
AM18(23)	4.31	5.27	6.6	7.17	6.32	5.87	7.46	7.06	6.21

To estimate the dye partition coefficients, experimental dependencies $\Delta I(C_L)$ were approximated by Eq. 7. The values of K_p were found to fall in the range $(1.1 - 3.8)\times10^3$, indicating that the examined compounds possess rather high lipid-associating ability (Table 1). At the next step of the study the resources of Virtual Computational Chemistry Laboratory

(http://www.vcclab.org) were employed to obtain the log*P* values for benzanthrone dyes in terms of different theoretical approaches. The results presented in Table 1 indicate that lipophilicity values significantly differ for various dyes. Particularly, for the groups 1 and 3 (except ABM) experimentally determined lipophilicities ($\log K_p$) appeared to be lower than calculated log*P* values, while the opposite relation between these parameters was found for the group 2. Since the benzanthrone part is identical for all of the examined compounds, the differences in lipophilicity could be ascribed to functionalities in their structure, *viz.*, amidino (group 2 and group 3) and amino group (group 1) in C-3 position, phenyl ring (AM12, A6, ABM, P9, P14), methyl (AM12, P9, A8, AM4(23)), ethyl (AM4 (23), AM21, AM15(23)), and bromine atom (group 3). In the case of ABM the main reason for relatively low log*P* values (compared to the other aminobenzanthrones of the group 1) is morpholino group in C-3 position, which probably decreases the calculated lipophilicity.

On the other hand, considerable differences are observed between lipophilicity values derived for the same dye using different theoretical approaches. This may be a consequence of the distinctions in the intrinsic computational algorithms. According to general classification, the approaches used for log*P* calculation can be divided into substructure-based and property-based methods [22]. There are two groups of substructure-based methods: fragment- and atom-based. In the fragment-based methods (AC log*P*, milogP) each structure is broken down into a set of substructural fragments and then the contribution of each fragment to the log*P* value of the entire set is assessed.

Alternatively, log*P* can be estimated with the use of the atom-based methods (ALOGP, MLOGP, XLOGP2, XLOGP3), in which lipophilicity calculation is based on breaking down the molecular structure into atomic fragments and using multiple regression to obtain the average contribution of each atom across the set [23]. The property-based methods (ALOGPs) employ the description of entire molecule and include either empirical methods based on 3D-structure or methods based on topological descriptors (ALOGPs) [18]. Notably, the KOWWIN lipophilicity was determined using both atom/fragment contribution methods of logP calculation, yielding differing log*P* values. Therefore, to assess lipophilicity prediction power of the above calculation procedures, the next step of the study was aimed at evaluating the extent of correlation between experimentally determined $\log K_p$ and calculated log*P* values parameters (Table 2).

Table 2. Correlation coefficients between experimentally determined ($\log K_p$) and calculated (log*P*) lipophilicity values for benzanthrone dyes

ALOGPs	**AC logP**	**miLOGP**	**ALOGP**	**MLOGP**	**KOWWIN**	**XLOGP2**	**XLOGP3**
Group 1							
0.785	0.756	0.758	0.696	0.76	0.876	0.795	0.724
Group 2							
0.617	0.796	0.725	0.81	0.737	0.619	0.799	0.846
Group 3							
0.916	-0.576	-0.603	-0.669	-0.689	0.749	-0.507	-0.609

Correlation analysis showed that generally $\log K_p$ correlates rather poorly with log*P* within each series (correlation coefficient was below 0.8 in most cases). The highest

correlation extent in the group 1 was found for KOWWIN (0.876), in the group 2 for XLOGP3 (0.846) and in the group 3 for ALOGPs (0.916). It can be assumed that the structure of substituent attached to benzanthrone molecule at C-3 position exerts influence on the lipophilicity of amino- and amidinobenzanthrones. On the other hand, attachment of bromine molecule to amidinobenzanthrone moiety seems to considerably affect the dye lipophilicities, since the highest prediction power was observed for property-based method (ALOGPs), only within the group 3, pointing to the importance of solute-solvent interactions as well as interactions between functional groups of solute molecule [24]. However, taking into account that in the reliable predictions correlation coefficients must be higher than 0.95, we can conclude that computational methods themselves not always can adequately prognosticate the extent of lipid bilayer partitioning of a certain dye. All the above considerations confirm the complex nature of interactions between the examined fluorescent dyes and model membranes, emphasizing the necessity of using a family of molecular descriptors for prediction of partition properties.

CONCLUSIONS

To summarize, the present study was undertaken to assess the predictive power of several commonly used computational approaches in determining the lipophilicity, expressed as log*P*, of a series of newly synthesized benzanthrone derivatives. Correlation analysis revealed that experimental partition coefficient $\log K_p$ weakly correlates with calculated lipophilicity log*P* of the examined compounds. The best agreement between calculated and experimental lipophilicities was observed for KOWWIN (group1), XLOGP (group2) and ALOGPs (group 3) methods, highlighting the importance of both structural and property-based factors in determining the lipophilic properties of benzanthrones.

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