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Newly synthesized benzanthrone derivatives as prospective fluorescent membrane probes

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ABSTRACT

Fluorescence spectral properties of a series of novel benzanthrone derivatives have been explored in lipid bilayers composed of zwitterionic lipid phosphatidylcholine (PC) and its mixtures with cholesterol (Chol) and anionic phospholipid cardiolipin (CL). Analysis of partition coefficients showed that all the examined compounds possess rather high lipid-associating ability, with the amidino derivatives exhibiting stronger membrane partitioning compared with the aminobenzanthrones. To understand how benzanthrone partition properties correlate with their structure, quantitative structure property relationship (QSPR) analysis was performed involving a range of quantum chemical molecular descriptors.

1. Introduction

Among a wide variety of fluorescent dyes currently used in biomedical research and industry, benzanthrone derivatives attract particular interest due to their favorable spectral properties, viz. large extinction coefficient, marked Stokes shift, negligible fluorescence in an aqueous phase, high sensitivity of fluorescence parameters to environmental polarity, etc. [1,2]. Bright color and intense fluorescence of benzanthrones gave the impetus to their use as disperse dyes for textiles, polymers, daylight fluorescent pigments and lasers [3,4]. It has been demonstrated that some 3-oxy- and 3-azomethine substituted benzanthrone derivatives, of yellow-green or orange-red color, exhibit features which qualify them as appropriate components of liquid-crystal (LC) systems for electro-optic displays of the "guest–host" type [5,6]. The absorption properties of 3-substituted benzanthrone dyes are determined by the charge transfer within chromophoric system, occurring between electron-donating groups in the C-3 position and electron-accepting carbonyl group [7,8]. It is known that spectral behavior of the dyes emitting from an intramolecular charge transfer (ICT) state is highly sensitive to the surrounding environment (polarity, viscosity, formation of hydrogen bonds or other intermolecular interactions) [9]. For this reason, ICT dyes represent effective microenvironmental sensors for monitoring structural changes in biological systems. In particular, benzanthrone derivatives were employed in DNA [10], protein [11] and membrane studies [12]. Furthermore, these dyes displayed pronounced sensitivity to the changes in immune status of a human organism at different pathologies [13,14].

Our recent study revealed high lipid-associating ability of a series of newly synthesized benzanthrone amino derivatives [12]. Moreover, spectral responses of the two examined dyes in different lipid systems proved to correlate with increased bilayer hydration. These findings allowed us to conclude that benzanthrone amino derivatives may be effective fluorescent probes for examining membrane-related processes, especially those coupled with the change in the degree of lipid bilayer hydration. In view of this it seemed of interest to extend our previous investigation to a new series of benzanthrone dyes (referred here as AM12, AM15, AM18, IAH, IBH and ISH), whose structures are given in Fig. 1. Our goal was three fold: (i) to obtain quantitative information about the dye partitioning into lipid phase of the model membranes composed of zwitterionic lipid phosphatidylcholine (PC) and its mixtures with cholesterol (Chol) and anionic phospholipid cardiolipin (CL); (ii) to assess benzanthrone sensitivity to the changes in physicochemical properties of lipid bilayer and (iii) to ascertain how benzanthrone partition behavior correlate with their structure and physicochemical properties via quantitative structure property relationship analysis (QSPR).

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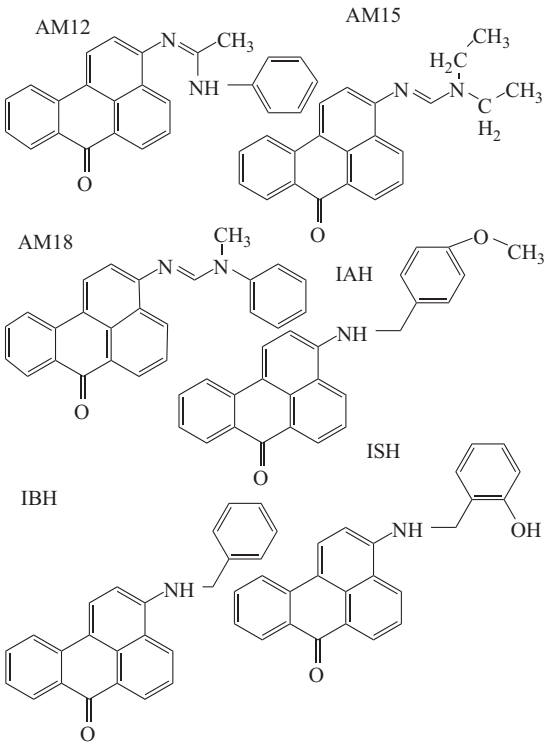

Fig. 1. Chemical structures of the examined benzanthrone dyes.

2. Materials and methods

2.1. Materials

Egg yolk phosphatidylcholine and beef heart cardiolipin were purchased from Biolek (Kharkov, Ukraine). Both phospholipids gave single spots by thin layer chromatography in the solvent system chloroform:methanol:acetic acid:water, 25:15:4:2, v/v. Chol was from Sigma. Benzanthrone dyes AM12, AM15, AM18, IAH, IBH and ISH were synthesized at the Faculty of Natural Sciences and Mathematics of Daugavpils University as described in detail elsewhere [15]. All other chemicals were of analytical grade and used without further purification.

2.2. Preparation of lipid vesicles

Unilamellar lipid vesicles composed of PC and its mixtures with (a) 5 or 10 mol% of CL or (b) 30 mol% of Chol were prepared by the extrusion method [16]. Thin lipid films were obtained by evaporation of lipids' ethanol solutions 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. Phospholipid concentration was determined according to the procedure of Bartlett [17]. The dye-liposome mixtures were prepared by adding the proper amounts of the probe stock solutions in ethanol to liposome suspension.

2.3. Fluorescence measurements

Steady-state fluorescence spectra were recorded with an LS-55 spectrofluorimeter (Perkin-Elmer Ltd., Beaconsfield, UK) at 20 °C using 10 mm path-length quartz cuvettes. Excitation wavelengths were 470 nm for AM12, 480 nm for AM15, 460 nm for AM18 and 520 nm for IAH, IBH and ISH. Excitation and emission slit widths were set at 10 nm. Fluorescence quantum yields were measured using rhodamine 101 as a standard. Absorption measurements were performed using an SF-46 spectrophotometer.

2.4. Calculations

Complete geometry optimization of the isolated molecules in the ground state was carried out using the semiempirical PM6 method. Quantum chemical calculations were performed with MOPAC 2012 Version10.006W-free academic license [18]. The energy of highest occupied molecular orbital (E_{HOMO}), the energy of lowest unoccupied molecular orbital (E_{LUMO}), cosmo area (*cosAr*), cosmo volume (molecular volume) (*cosVol*) and molecular length (L), the charge on nitrogen atom at the C-3 position $q(N)$ and the total charge on carbon atoms $\sum q(C)$ were extracted directly from the data files following the geometry optimization. The dipole moments of the ground (μ) and excited (μ_e) states were calculated using ab-initio method at B3LYP/6-31G level of theory with GAMESS 11.

Virtual Computational Chemistry Laboratory (http:/www.vcclab.org) was used for calculation of lipophilicity of the examined compounds.

3. Theory

Total concentration of the dye distributing between the 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 two phases (K_P) is defined as [19]

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

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

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

where a_f and a_L represent molar fluorescence of the dye free in solution and in the lipid environment, respectively. From 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 the 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 ith bilayer constituent, v_i is its molecular volume taken as 1.58 nm^3, 3 nm^3 and 0.74 nm^3 for PC, CL and Chol respectively [20]. For Chol-containing systems condensing effects of this lipid was taken into account, so that the above v value for PC was reduced by the factor 1.3.

The relationship between K_p and fluorescence intensity increase (ΔI) upon the dye transfer from water to the lipid phase