European Journal of Medicinal Chemistry 76 (2014) 79–86

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis of coumarin derivatives as fluorescent probes for membrane and cell dynamics studies

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ARTICLE INFO

Article history:
Received 9 August 2013
Received in revised form
13 November 2013
Accepted 8 February 2014
Available online 11 February 2014

Keywords:
Coumarins
Probes
Cell membranes
Molecular dynamics

ABSTRACT

Three coumarin-derived fluorescent probes, 3-acetyl-7-[(6-bromohexyl)oxy]-2*H*-chromen-2-one (**FM1**), 7-[(6-bromohexyl)oxy]-4-methyl-2*H*-chromen-2-one (**FM2**) and ethyl 2-{7-[(6-bromohexyl)oxy]-2-oxo-2*H*-chromen-4-yl}acetate (**FM3**), are described, with their photophysical constants. The compounds were tested in preliminary studies employing epifluorescence microscopy demonstrating that they allow the imaging of human neuroblastoma SH-SY5Y cell membranes. The structure of **FM3** was confirmed by X-ray crystallographic analysis. Molecular dynamics (MD) simulations were used to characterize the localization and interactions of the studied compounds with a lipid bilayer model of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC).

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1. Introduction

A biomembrane is a dynamic structure that acts as the primary barrier isolating the cell cytosol from the extracellular medium or separating distinct cell compartments [1]. Its fundamental structure is a phospholipid bilayer presenting at least two distinct chemical regions, a hydrophobic region inside the membrane and a hydrophilic region distributed between the interfaces with the cytosol and the cell exterior or the interior of specific intracellular structures [2]. A main function of biological membranes is to ensure the integrity of the cell itself or of certain organelles, and to control the functions of membrane proteins [3–6]. Cell membranes are critical for communication with the outer world by enabling the transfer of many compounds important for cell metabolism and for chemical and electrical signaling [7]. This function requires the correct assembly of various molecular structures in and around the cell membrane including receptors, transport proteins and specialized membranes [8]. For many years lipids were viewed as randomly organized building blocks of biological membranes. This interpretation was adopted from the Singer and Nicholson fluid mosaic model, proposed in 1972 [9]. However, in the 1990s, this was gradually superseded by the raft hypothesis, which proposed a laterally segregated distribution of lipid molecules [10–12]. Information on structures and processes occurring at different depths in a membrane has been obtained using fluorescent or photoactive probes. Both techniques are based on the fact that the membrane hydrophobic core incorporates nonpolar lipid fatty acyl chains, and a fluorescent or photoactive chromophore can be attached to the nonpolar moieties to increase its affinity for the core region [13]. Numerous fluorescent probes have been used that partition into the membrane hydrophobic region, e.g. diphenylhexatriene and anilinonapthalene sulfonic acid. However, the use of such probes only provides average information on the nature of the membrane. In order to get depth-dependent information, a practical approach has been to attach such fluorescent probes to fatty acids at distinct positions so that the membranes can be probed at different depths [14,15]. Fluorescence microscopy is widely used for studying the organization and dynamics of membranes [16,17]. The advantages of this technique include high sensitivity and time resolution, multiple measurable parameters yielding complementary information, and spatial resolution. However, the use of fluorescent probes in membrane studies can potentially cause significant perturbations in membrane

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http://dx.doi.org/10.1016/j.ejmech.2014.02.016
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structure, dynamics and thermotropic behavior [18–22]. Molecular dynamics (MD) simulations, by providing detailed atomic-scale information on phospholipid bilayers [23,24], represent a valuable complement to these studies by detecting and quantifying the magnitude of the perturbations induced by molecular probes in the host lipid structure.

We have now shown that three new coumarin-derived fluorescent probes are useful to image the plasmalemma of cultured human neuroblastoma cells, and obtained a more detailed picture of this finding by determining the crystal structure of one of these probes and characterizing the localization and interactions of the studied compounds with a lipid bilayer model by MD simulations.

2. Results and discussion

Chemistry. Fluorescent probes **FM1**, **FM2** and **FM3** were designed to provide appropriate lipophilicity while also allowing for further derivatization by substitution of the terminal bromine atom. The route employed is summarized in Scheme 1 (Supporting Information). The compounds were obtained starting from resorcinol, which was formylated using the Vilsmeier–Haack reaction [37]. Knoevenagel condensation of the aldehyde intermediate (**1**) with ethyl acetoacetate afforded hydroxycoumarin **2** [25], while compound **3** was synthesized through Pechmann condensation of **1** with citric acid and was subsequently esterified to obtain compound **4** [26]. Reaction of 7-hydroxycoumarins **2**, **3** and **4**, in a Williamson-type reaction with 1,6-dibromohexane, gave the corresponding ethers **FM1**, **FM2** and **FM3** [38]. Unexpectedly, the formation of **FM2** was attended by decarboxylation of coumarin **2** in the presence of potassium carbonate, giving the 4-methylcoumarin. Scheme 2 (Supporting Information) shows a possible mechanism for the K-catalyzed protiodecarboxylation. Similar mechanisms have been reported using the decarboxylating agent $AgCO_3$ [39–42], but the present result suggests that expensive silver salts are not necessary to effect this reaction.

Perspective views of the crystal structure of ethyl 2-(7-(6-bromohexyloxy)-2-oxo-2*H*-chromen-4-yl)acetate (**FM3**) with its atom labels are depicted in Fig. 1. The crystal data, data collection and refinement are summarized in Table 1.

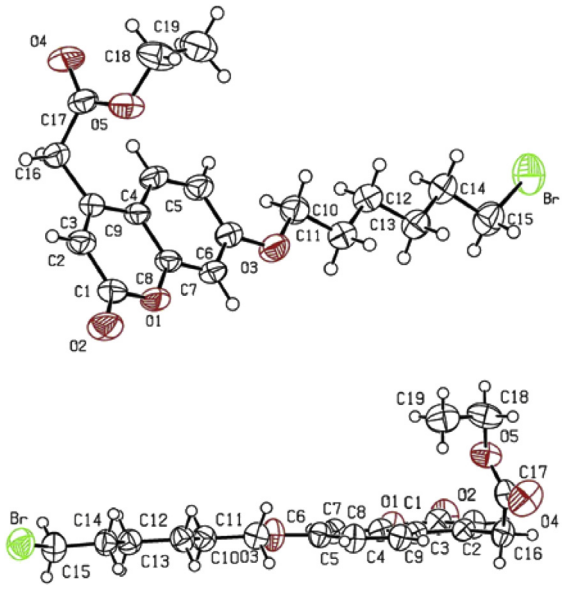

Fig. 1. Mutually approximately perpendicular views of the structure of **FM3** showing the atom numbering scheme. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary radii.

Table 1
Crystal data and details of the structure determination for **FM3**.

Compound	$C_{19}H_{23}O_5Br$
Formula weight	411.28
Crystal shape/color	Block/colorless
Crystal size (mm)	0.10 × 0.12 × 0.30
Crystal system/space group	Triclinic/P-1
a (Å)	7.7862 (13)
b (Å)	10.2539 (16)
c (Å)	12.105 (3)
α (°)	87.963 (18)
β (°)	77.255 (18)
δ (°)	85.625 (18)
V (Å^3)	939.7 (3)
Z	2
Dcalc (g/cm^{-3})	1.454
Wavelength, Mo Kα (Å)	0.71073
T (K)	293 (2)
F (000)	424
θ-range (°)	$1.73 < \theta < 25.00$
hkl-range	−8: 9, −8:12, −14:14
μ (mm^{-1})	2.212
Reflections collected/R_{int}/R_σ	7225/0.0209/0.0289
Reflections unique/parameters	2499/231
R, *wR2* [$F^2 > 2\sigma(F^2)$]	0.0337, 0.0757
R, *wR2* (all reflections)	0.0501, 0.0838
Goodness-of-Fit on F^2 (GooF = S)	1.025
Residual electron density $\Delta\rho_{max}/\Delta\rho_{min}$ (e Å^{-3})	0.421/−0.428

In **FM3**, the coumarin ring and the aliphatic chain at C6 (the 6-bromohexyloxy substituent) are practically coplanar, with a C10–O3–C6–C5 torsion angle value of 3.1(4)° (Fig. 1). The hexyloxy chain (O3/C10–C15) is essentially flat with average mean deviation of 0.0110 Å from the least-squares plane (where the maximum deviation from the plane is −0.0205 Å for C15). The halogen atom lies in the mean plane of the zigzagging aliphatic chain [C13–C14–C15–Br = −176.00(18)°], while in 7-((6-bromohexyl)oxy)-4-methyl-2-oxo-2*H*-chromene the bromine atom departs strongly from this plane, with C13–C14–C15–Br = 65.5(4)° [43]. The ethyl acetate substituent group (at C3) in **FM3** is strongly tilted out of the mean plane of the coumarin ring (Fig. 1, bottom), with a C7–C16–C3 angle of 115.9(2)° [C2–C3–C16–C17 = 115.1(3)°]. All the other relevant structural parameters (bond distances and angles) are as expected and in acceptable agreement with the recently described analogues [43–47].

The intermolecular contacts are responsible for the three-dimensional architecture in the crystal packing of **FM3** [48–50]. The antiparallel stacked molecules have π–π interactions with a *Cg1···Cg1′* distance of 3.6811(16) Å (Fig. S1; Supporting Information, top). The intermolecular C4–H4···O4 interactions lead to the formation of dimers parallel to [001], which can be described as a graph-set descriptor $R_2^2(14)$ ring (Fig. S1; Supporting Information, bottom). The H···O contact distance is 2.50(2) Å [>*D*–H···*A* = 127.5(19)°] and the C···O contact distance is 3.171(3) Å.

Spectral characteristics of **FM1**, **FM2** and **FM3** such as the absorption maxima (λ_{ab}), emission maxima (λ_{em}), molar extinction coefficient (ε), and quantum yield (Φ), were measured in a mixture of ACN and aqueous 20 mM HEPES buffer, pH 7.4, 1:1. Complete data are presented in Table 2. The electronic absorption spectra of probes **FM1** – **FM3** displayed absorption maxima in the region

Table 2
Photochemical properties of **FM1**, **FM2** and **FM3**.

Compounds	λ_{ab} (nm)	λ_{em} (nm)	ε Mol^{-1} dm^{-3} cm^{-1}	Φ	Stokes shift
FM1	360	420	19,786	0.0041	3739
FM2	320	375	13,875	0.259	4584
FM3	310	460	17,703	0.0095	10,819

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