Supporting Information

Interactions of Marine-Derived γ-Pyrone Natural Products with Phospholipid Membranes

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Materials

Phosphatidylcholine (from egg yolk) was purchased from Lipid Products as a solution in chloroform:methanol. Di-8-ANEPPS was purchased from Invitrogen. Cholesterol (≥99%) and DMSO (BioReagent for molecular biology, ≥99.9%) were purchased from Sigma Aldrich. 100 nm pore polycarbonate filters were purchased from Nucleopore Filtration Products. The pressure extruder was purchased from Lipex Biomembranes. Molecules 1–3 were synthesised by a method previously described.¹

Methods

Liposome preparation and labelling

Unilamellar phospholipid membrane vesicles were prepared using a pressure extrusion technique. Phosphatidylcholine (PC) and cholesterol (where appropriate) were added to a round bottomed flask and the solvent removed under a stream of oxygen-free nitrogen. The resulting thin lipid film was rehydrated to a concentration of 13 mM using 10 mM Tris pH 7.4 buffer, giving a suspension of multilamellar vesicles. This solution was subjected to five freeze-thaw cycles using liquid nitrogen, creating unilamellar vesicles which were then extruded ten times through polycarbonate filters with a 100 nm pore size at 45 °C, giving a monodisperse, unilamellar suspension of phospholipid vesicles (PLVs).

PLVs were labelled by adding di-8-ANEPPS (1mg/mL solution in EtOH; 15 µL of this solution was used per mL of liposome solution) to a 13 mM solution of liposome. The mixture was incubated at 37 °C for 1 h in the dark prior to use in fluorescence measurement experiments.

Fluorescence measurements

Fluorescence spectra and dual-wavelength ratiometric measurements were recorded at 25 °C using a HORIBA Jobin Yvon Fluoromax-4 spectrofluorometer. For dual-wavelength
recordings, the time-dependent ratio of fluorescence R(460 nm/520 nm) was obtained by exciting the samples at 460 nm and 520 nm, and measuring the intensity ratio at a fixed emission of 590 nm, whilst aliquots of compound (33 mM in DMSO) were titrated into labelled PLVs (400 µM) in a continuously stirred cuvette. Control experiments were conducted by titrating equivalent volumes of DMSO, and subtracting any signal from that obtained upon compound titration. The fluorescence excitation spectrum of di-8-ANEPPS was recorded prior to and upon completion of each titration experiment. Fluorescence difference spectra were obtained by subtracting the former spectrum from the latter.

**Fixed-angle light scattering**

Samples containing increasing quantities of compound made up to 15 µL with DMSO were made up to a volume of 2 mL using either 10 mM Tris pH 7.4 buffer or a solution of PLVs in buffer so that the total concentration of PLVs in the sample was 400 µM. Each sample was incubated for 1 h at 25 °C in the dark. The intensity of light scattering of each sample at an angle of 90° was then measured using a HORIBA Jobin Yvon Fluoromax-4 spectrofluorometer, using a 600 nm irradiation wavelength and a 2 nm bandpass.

**UV Spectra**

UV spectra (methanol) were measured using a Philips PU 8720 Series UV/Vis Scanning Spectrophotometer.

**UV Spectrum of (2E,4E,6E)-polyene 1**
UV Spectrum of (2Z,4E,6E)-polyene 2
UV Spectrum of tridachiahydropyrone (3)

HPLC analysis was run using an analytical Chiral Cel OD-H column, with a solvent system of hexane:IPA::99.5:0.5; flow rate = 0.5 mL/min; temperature = 25 °C; injection volume = 10 µL. Retention time: 1st eluted (-)-3 = 8.41 min; 2nd eluted (+)-3 = 9.89 min. 1st eluted: $[\alpha]_{D}^{23} = -225.6$ (c=0.42 g/100 cm$^3$, CHCl$_3$) [isolated natural product: $[\alpha]_{D} = -476.1$ (c=0.49, CHCl$_3$)]; 2nd eluted: $[\alpha]_{D}^{23} = +190.4$ (c=0.08 g/100 cm$^3$, CHCl$_3$) (90% ee by HPLC).

Racemic tridachiahydropyrone ((±)-3)
1\textsuperscript{st} eluted enantiomer (-)-3

2\textsuperscript{nd} eluted enantiomer (+)-3

Binding Data

PC100 and PC70Chol30 PLVs were stained with the potentiometric indicator di-8-ANEPPS. The ratio of fluorescence, R, excited at 460 nm/520 nm was recorded as a function of time as aliquots of compound in DMSO were titrated in. An example of the time-dependent data collected is given below (Figure S1), exemplifying the effect of compound addition on R versus a DMSO control.

Figure S1. Effect of 82.5 μM 2 on R(460 nm/520 nm), and effect of equivalent volume of DMSO.
Binding profiles were then created by plotting the change in R against compound concentration (Figure S2).

![Diagram](image)

**Figure S2.** Binding profile of 1-3 with A: PC100 PLVs; B: PC70Chol30 PLVs. Dotted lines mark CMC for 1 (black line) and 2 (blue line).

**Critical Micelle Concentration**

Plotting the change in light scattering at a fixed angle of 90° against increasing compound concentration enabled determination of the critical micelle concentration (CMC). At concentrations below CMC light scattering did not differ significantly from background scattering. At concentrations above CMC the observed scattering increased significantly. These two sets of datapoints were separately fitted to linear equations, and the point of intercept taken as CMC. Figure S3 demonstrates this method of CMC determination.
Figure S3. CMC determination for 1 in presence of PC100 PLVs.

The point of CMC was determined for compounds 1 and 2 in both the presence and absence of PC100 and PC70Chol30 PLVs. The results are outlined below (Figure S4).

Figure S4. Critical Micelle Concentrations (CMCs) for 1 and 2.

Superimposing these CMC values upon the binding profiles (Figure S2), it is clear that the disruption to the profiles of 1 and 2 coincides with the aggregation of the molecules into micelles. Thus for these two molecules the post CMC datapoints were omitted before further manipulation of the data was conducted.

Fitting of Binding Data

The best fitting model of equations 1 and 2 for each binding profile was determined using an extra sum-of-squares F test (GraphPad Prism v5.0).
Null hypothesis: eqn 1  \( y = (B_{max} x) / (K_d + x) \)

Alternative hypothesis: eqn. 2  \( y = (B_{max} x^n) / (K_d^n + x^n) \)

\[ F = \frac{SS_1}{SS_2} \]

Where  \( SS_1 = \text{Sum of squares (null)} \)

\( SS_2 = \text{Sum of squares (alt.)} \)

\( DF_1 = \text{Degrees of freedom (null)} \)

\( DF_2 = \text{Degrees of freedom (alt.)} \)

Null hypothesis accepted for P values \( \geq 0.05 \)

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<th>Compound</th>
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*Table S1*. Statistical Parameters. \(^a\)DF<sub>n</sub>=(DF<sub>1</sub>-DF<sub>2</sub>), \(^b\)DF<sub>d</sub>=DF<sub>2</sub>, \(^c\)For PC70Chol30 Eqn. 2 best fit as eqn. 1 fit deemed ambiguous.

**Equilibrium Geometries**

The equilibrium geometries of 1 and 2 were calculated using Spartan ‘10 Version 1.1.0.

Geometry Optimisation was performed using the B3LYP density functional model with a 6-31G* basis set.

*(2E,4E,6E)-polyene 1*
(2Z,4E,6E)-polyene 2
References

