Supporting Information

Bilayer Interaction and Protein Kinase C-C1 Domain binding studies of Kojic Acid Esters

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Table of Content

Supporting Information

(I) Characterization of the synthesized compounds:

2-(hydroxymethyl)-4H-pyran-4-one octanoate (1): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (S, 1H, ArH), 6.47 (S, 1H ArH), 4.86 (S, 2H, -OCH_2), 2.38 (t, 2H, J = 8 Hz, -OCH_2 (ester)) 1.65-1.61 (m, 2H, -CH_2), 1.27-1.23 (m, 8H), 0.84 (t, 3H, J = 5.6 Hz, -CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.2, 172.9, 163.3, 146.1, 138.2, 111.3, 61.4, 29.8, 29.3, 29.1, 29.0, 25.0, 14.3; HRMS (ESI) Calcd for $C_{14}H_{21}O_5$ $[M+H]^+$ 268.1311, Found: 269.1387.

2-(hydroxymethyl)-4H-pyran-4-one laurate (2): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (S, 1H, ArH), 6.52 (S, 1H ArH), 4.45 (S, 2H, OCH_2), 2.55 (t, 2H, J = 8 Hz, -OCH_2 (ester)) 1.70-1.67 (m, 2H, -CH_2), 1.35-1.22 (m, 16H), 0.84 (t, 3H, J = 6.8 Hz, -CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.4, 171.2, 168.4, 148.0, 141.2, 113.3, 60.9, 33.8, 32.1, 29.9, 29.8, 29.6, 29.5, 29.4, 29.2, 24.9, 22.9, 14.3; HRMS (ESI) Calcd for $C_{18}H_{29}O_5$ $[M+H]^+$ 325.2016, Found: 325.2020.

2-(hydroxymethyl)-4H-pyran-4-one palmitate (3): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (S, 1H, ArH), 6.53 (S, 1H, ArH), 4.44 (S, 2H, -OCH_2), 2.56 (t, 2H, J = 8 Hz, -OCH_2(ester)) 1.7-1.67 (m, 2H, CH_2), 1.36-1.23 (m, 24H), 0.85 (t, 3H, J = 8 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 171.3, 169.0, 148.1, 141.2, 113.2, 60.83, 33.87, 32.16, 29.93, 29.89, 29.85, 29.68, 29.60, 29.47, 29.23, 24.94, 22.93, 14.37; HRMS (ESI) Calcd for $C_{22}H_{37}O_5$ $[M+H]^+$ 381.2636, Found: 381.2640.

2-(hydroxymethyl)-4H-pyran-4-one stearate (4): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (S, 1H, ArH), 6.53 (S, 1H, ArH), 4.45 (S, 2H, -OCH_2), 2.56 (t, 2H, J = 8 Hz, -OCH_2(ester)) 1.71-1.68 (m, 2H, -CH_2), 1.36-1.23 (m, 28H), 0.85 (t, 3H, J = 6.8 Hz, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 171.2, 168.4, 148.0, 141.2, 113.3, 60.91, 33.9, 32.1, 29.9, 29.8, 29.7, 29.6, 29.4, 29.2, 24.9, 22.9, 14.3; HRMS (ESI) Calcd for $C_{24}H_{41}O_5$ $[M+H]^+$ 409.2949, Found: 409.2975.

2-(hydroxymethyl)-4H-pyran-4-one oleate (5): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (S, 1H, ArH), 6.56 (S, 1H ArH), 5.34 (m, 2H, -CH═CH-), 4.47 (S, 2H, -OCH_2), 2.58 (t, 2H, J = 8 Hz, -OCH_2 (ester)), 2.0-1.99 (m, 4H), 1.73-1.67 (m, 2H, -CH_2), 1.38-1.19 (m, 20H), 0.87 (t, 3H, J = 6.8 Hz, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 171.1, 168.8, 148.0, 141.2, 130.2, 129.9, 113.2, 60.8, 33.8, 32.1, 29.95, 29.9, 29.7, 29.3, 29.27, 29.15, 28.6, 27.4, 26.49, 25.4, 24.9, 22.7, 14.3; HRMS (ESI) Calcd for $C_{24}H_{39}O_5$ $[M+H]^+$ 407.2792, Found: 407.2849.

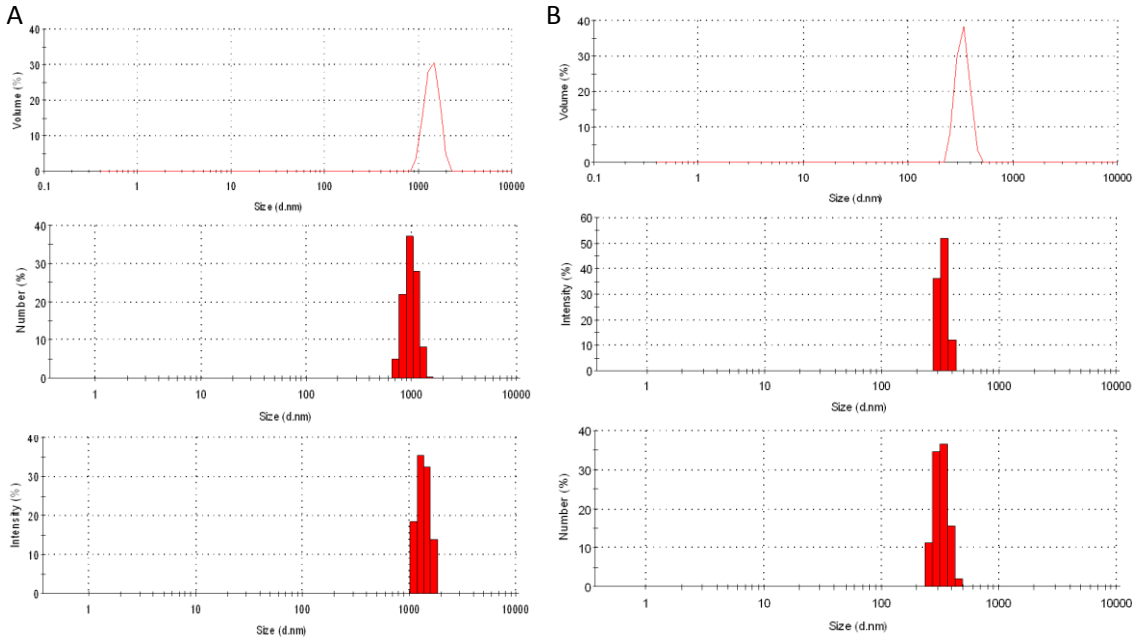
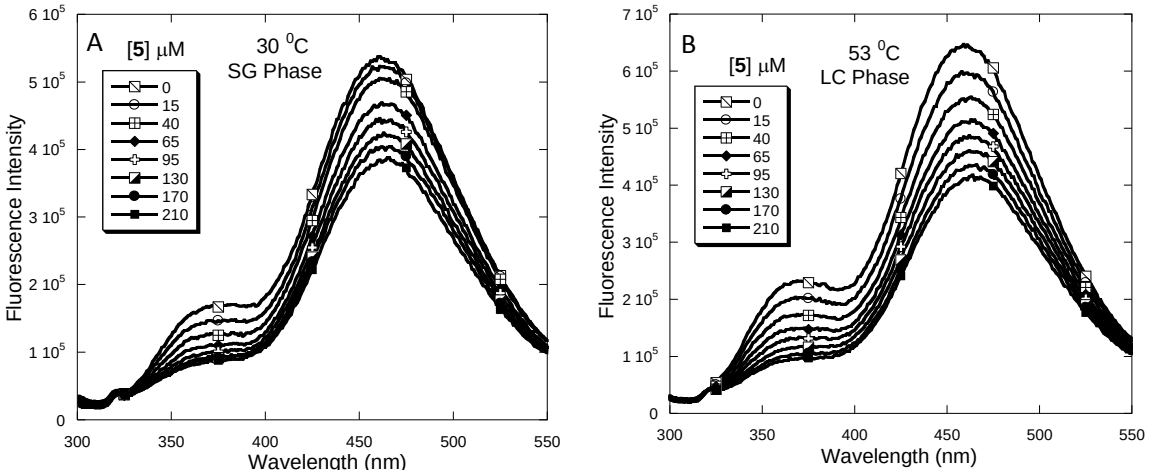


Figure S1: Size distribution of the compounds in aqueous solution (PBS buffer pH 7.0) at 25 °C for (A) 120 μM of **4** at *t* = 5 min, and (B) 150 μM of 5 at *t* = 5 min.



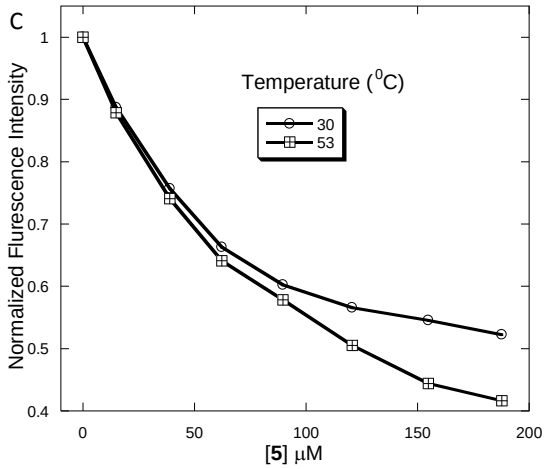


Figure S2: Fluorescence intensity measurements of 1-naphthol in the presence of DPPC vesicle. Emission spectra of 1-naphthol (λ_{ex} = 290 nm) with increasing concentration of kojic acid ester **5** at (A) 30 ^{0}C (SG phase) and (B) 53 ^{0}C (LC phase) in DPPC vesicles. Change in NpOH* fluorescence intensity (at 370 nm) with the increase in compound **5** concentration at 30 ^{0}C and 53 ^{0}C in DPPC vesicles. [DPPC] = 0.2 mM, [1-napthol] = 4 μM.

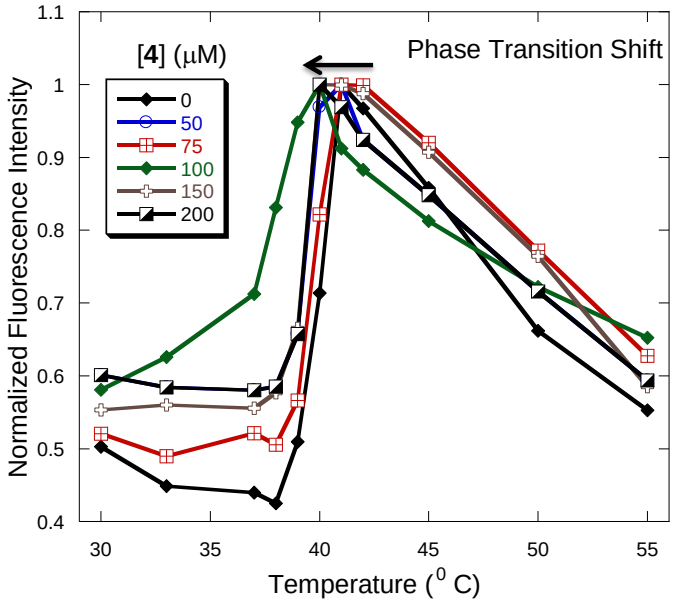
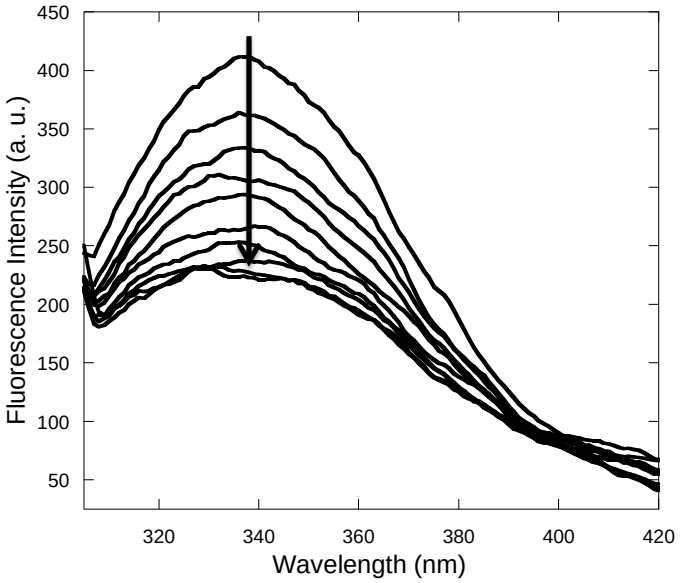
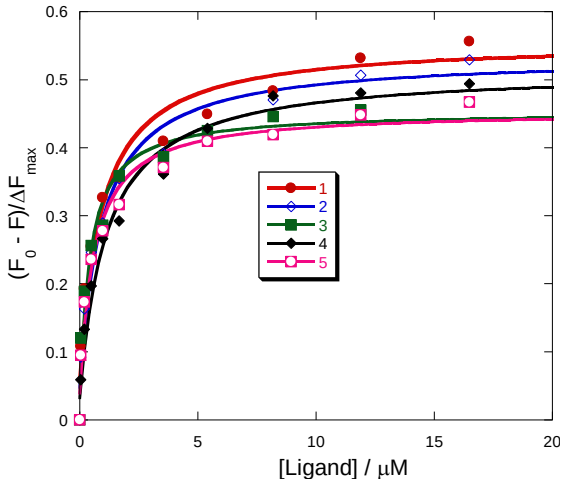


Figure S3: Effect of compound **4** on the phase transition temperature of DPPC vesicle. Change in NpOH* fluorescence intensity with the increase in compound **4** concentration at different temperatures in DPPC vesicles. λ_{ex} = 290 nm, λ_{em} = 370 nm, [DPPC] = 0.2 mM, [1-napthol] = 4 μM.



(II) Figure S4: Representative quenching plot of PKCθ-C1b fluorescence by ligand. Addition of increased concentration of compound **3** (0-22 μM**)** to PKCθ C1b (1 μM) quenched the intrinsic fluorescence intensity.

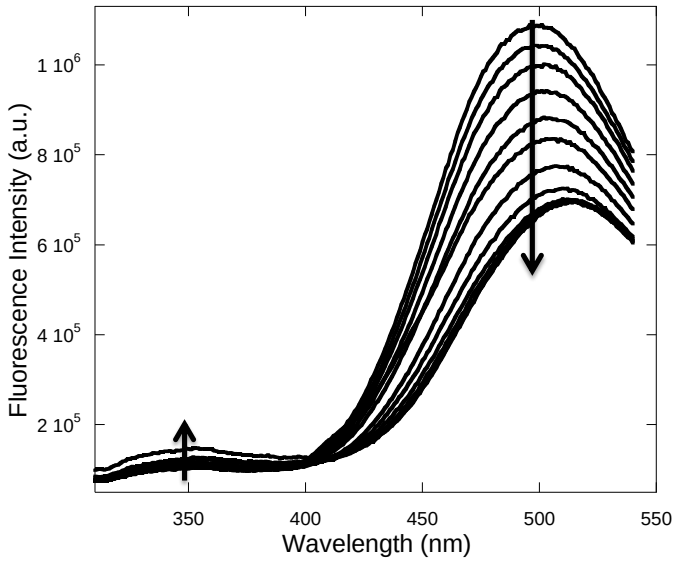


(III) Figure S5: Binding isotherms of ligands with PKCθ-C1b subdomains. Representative plot of fluorescence intensity of PKCθ-C1b subdomains (1 μM) in buffer (20 mM Tris, 160 mM NaCl, 50 μM $ZnSO_4$, pH 7.4) in the presence of varying concentration of compounds **1-5**, where F and F_0 are fluorescence intensity in the presence and absence of the ligands, respectively. The solid lines are nonlinear least squares best fit curves

(IV) Table S1. Anisotropy[a] values of the ligands in the presence and absence of the PKCδ- and PKCθ-C1b proteins at room temperature.

Compound	PKCδ C1b	PKCθ C1b
buffer[b]	0.0746 (0.00070)	0.0682 (0.02220)
DAG_8[c]	0.1493 (0.06160)	0.2372 (0.07740)
1	0.1414 (0.04490)	0.2234 (0.08780)
2	0.2406 (0.02404)	0.2967 (0.03950)
3	0.2415 (0.01165)	0.2502 (0.01060)
4	0.2589 (0.01270)	0.3016 (0.05243)
5	0.3206 (0.05246)	0.3192 (0.07474)

[a)]Values in parenthesis indicate standard deviations.
[b)]Protein, 1 μM in buffer (20 mM Tris, 160 mM NaCl, 50 μM $ZnSO_4$, pH 7.4).
[c)]Ligands**,** 10 μM; protein, 1 μM in buffer (20 mM Tris, 160 mM NaCl, 50 μM $ZnSO_4$, pH 7.4)



(V) Figure S6: Representative protein-to-membrane FRET assessment under liposomal environment. Addition of increased concentration of DAG_8 (0 - 80 μM) to PKCδ-C1b sundomain (1 μM) bound to the active liposome (PC/PE/dPE/ligand (75/15/5/5)) of compound **5** decreases the FRET signal at 495 nm. All the measurements were performed in 20 mM Tris, pH 7.4 containing 160 mM NaCl and 50 μM $ZnSO_4$.