

# Supplementary materials

## *Physicochemical Characterization and In Vitro Biological Evaluation of TPGS-Stabilized Cationic Nanoliposomes for 8-Bromo-6-Chloroflavone Delivery*

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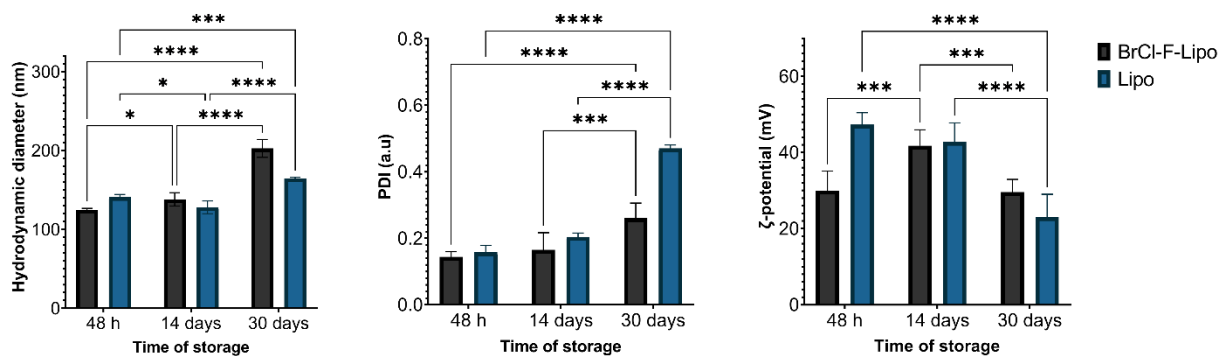
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<i>Time of storage</i>	<i>D<sub>h</sub> (nm)</i>	<i>PDI</i>	<i>ζ-potential (mV)</i>
<i>48 h</i>	125.06 ± 1.69	0.153 ± 0.009	+28.14 ± 3.32
<i>14 days</i>	138.24 ± 8.36	0.16 ± 0.05	+41.8 ± 4.15
<i>30 days</i>	202.86 ± 11.31	0.26 ± 0.04	+29.27 ± 3.72

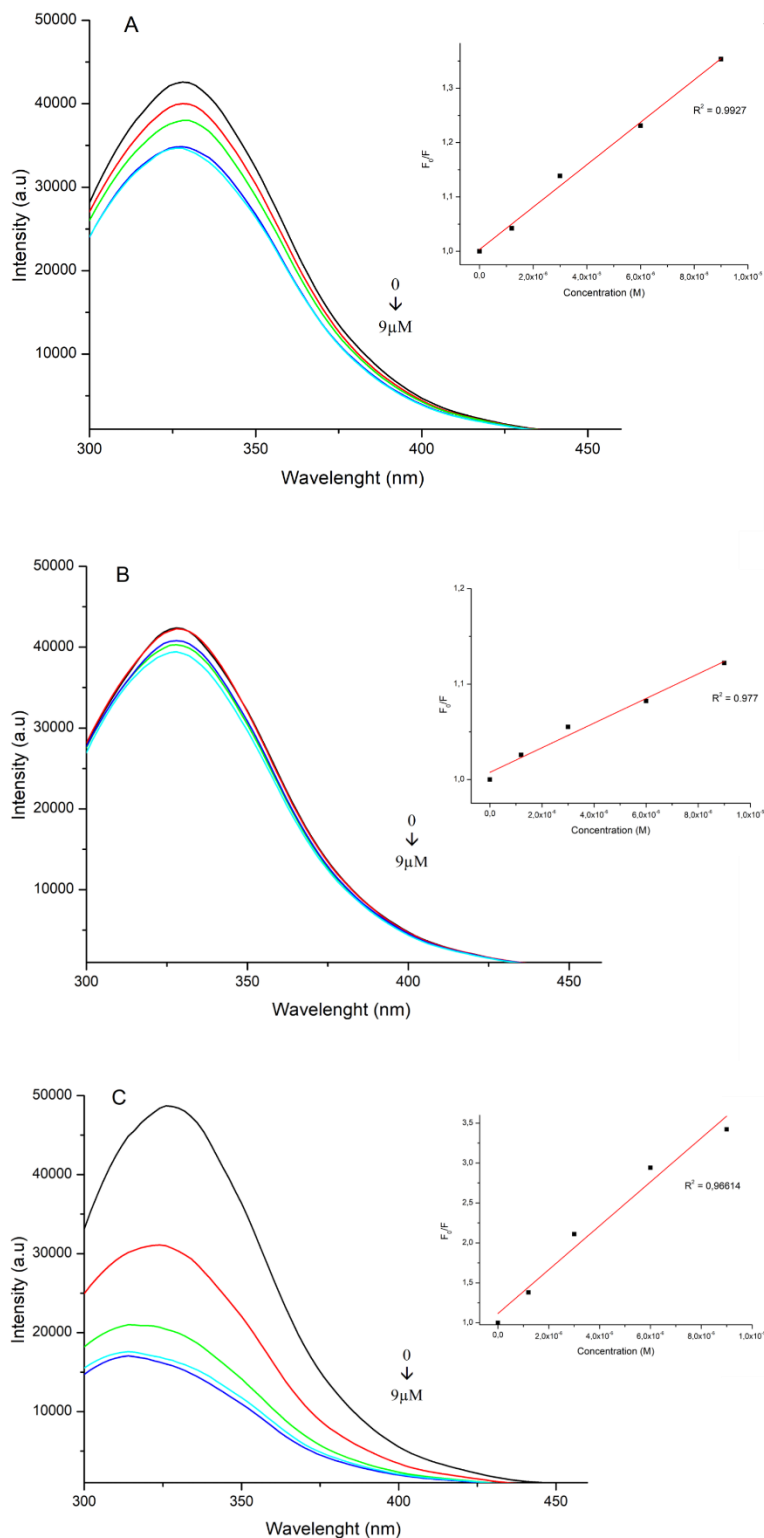
**Tab.S1** Hydrodynamic diameter, polydispersity index and zeta potential of BrCl-F-Lipo measured after 48 h, 14 days and 30 days. Mean ± standard deviation (n=5).

<i>Time of storage</i>	<i>D<sub>h</sub> (nm)</i>	<i>PDI</i>	<i>ζ-potential (mV)</i>
<i>48 h</i>	141.04 ± 3.30	0.15 ± 0.02	+47.32 ± 3.13
<i>14 days</i>	128.1 ± 8.16	0.20 ± 0.01	+42.8 ± 4.99
<i>30 days</i>	164.72 ± 1.32	0.47 ± 0.01	+23.03 ± 6.00

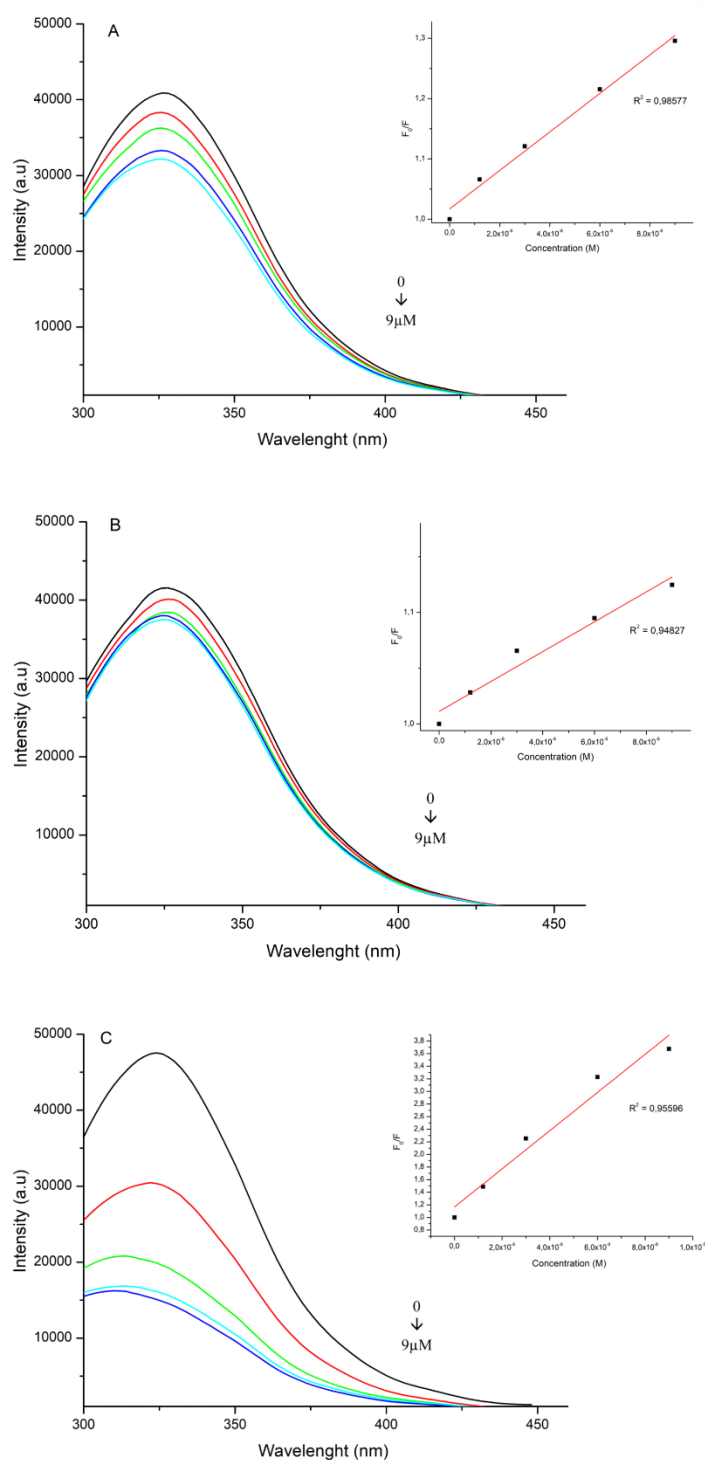
**Tab.S2** Hydrodynamic diameter, polydispersity index and zeta potential of Lipo measured after 48 h, 14 days and 30 days. Mean ± standard deviation (n=5).



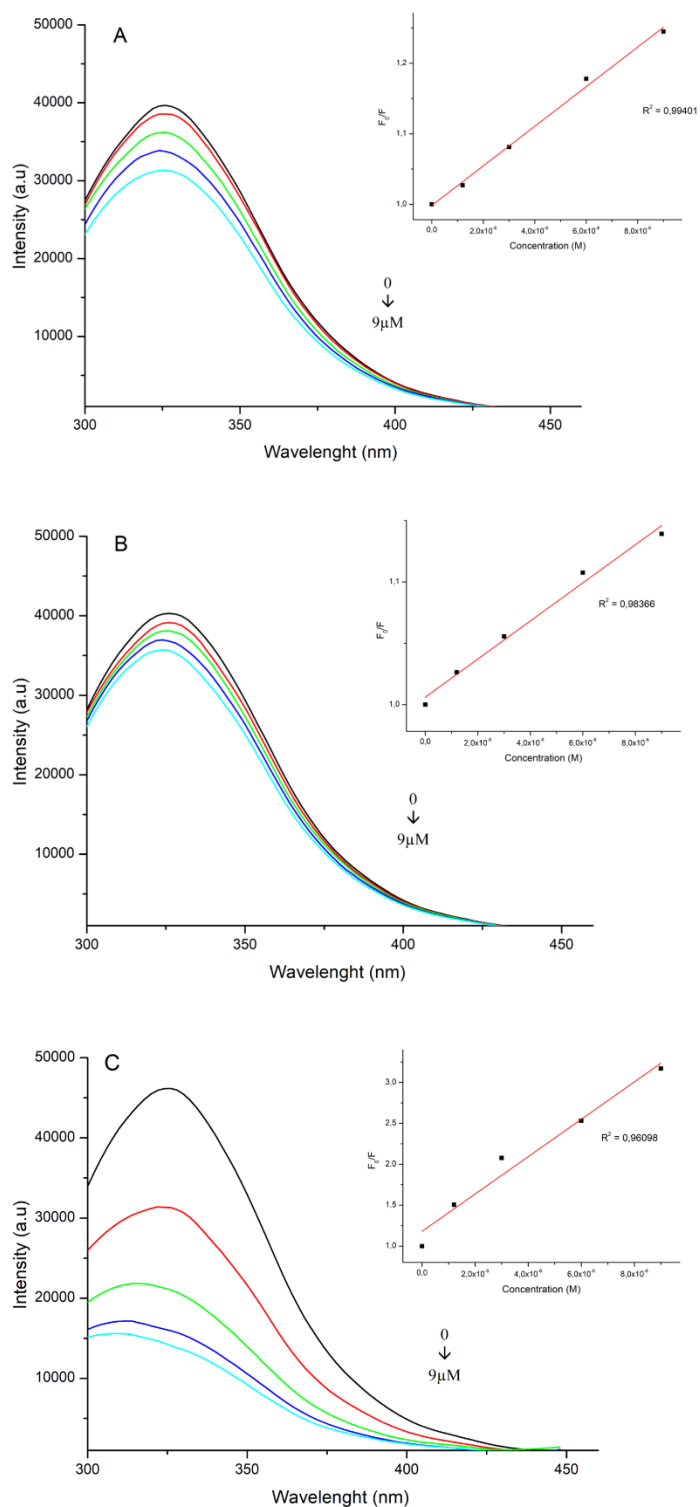
**Fig.S1** Hydrodynamic diameter, polydispersity index (PDI) and zeta potential ( $\zeta$ -potential) of BrCl-F-Lipo and Lipo measured after 48 h, 14 days and 30 days. Error bars represent standard deviation (n = 5). Statistical significance vs. control: \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p < 0.0001.



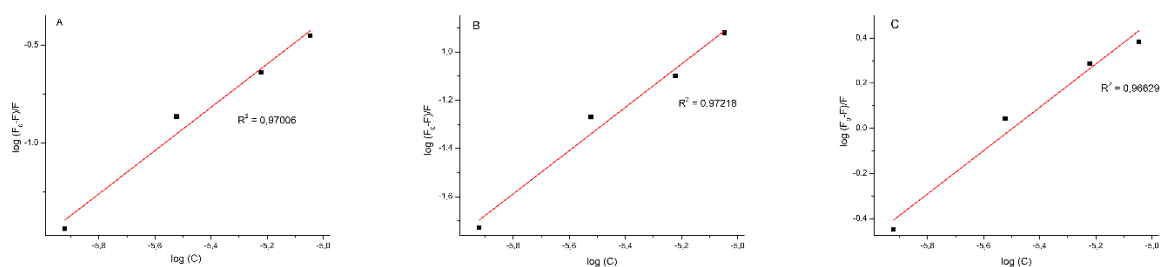
**Fig. S2** Emission spectra of HSA in the presence of various concentrations and Stern-Volmer plots of  $F_0/F$  against concentration of (A) BrCl-F-Lipo (B) Lipo and (C) BrCl-F. Control is marked black and consecutive spectra of the studied compounds (marked color) are in the following concentrations 1.2, 3, 6 and 9  $\mu\text{M}$ ,  $\lambda_{\text{ex}} = 280 \text{ nm}$ ,  $T = 295 \text{ K}$ . Spectra smoothed with SG 15 (see Method).



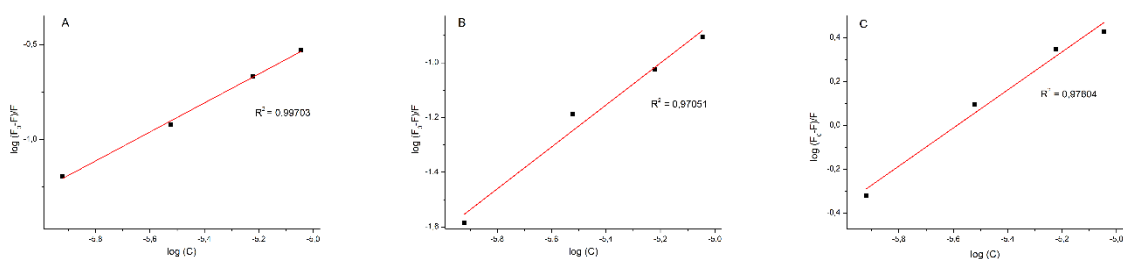
**Fig. S3** Emission spectra of HSA in the presence of various concentrations and Stern-Volmer plots of  $F_0/F$  against concentration of (A) BrCl-F-Lipo (B) Lipo and (C) BrCl-F. Control is marked black and consecutive spectra of the studied compounds (marked color) are in the following concentrations 1.2, 3, 6 and 9  $\mu$ M,  $\lambda_{ex} = 280$  nm,  $T = 300$  K. Spectra smoothed with SG 15 (see Method).



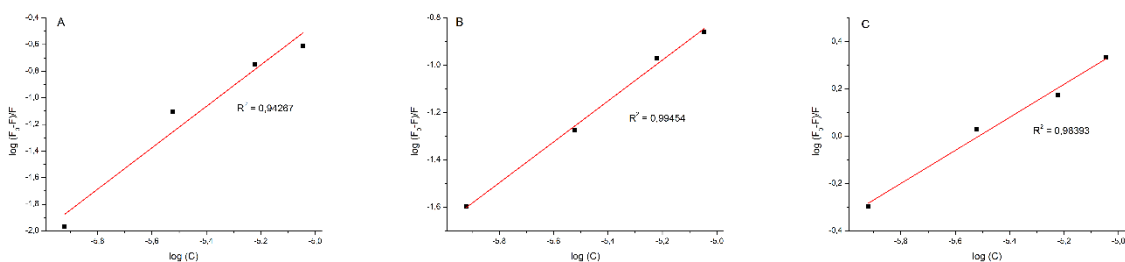
**Fig. S4** Emission spectra of HSA in the presence of various concentrations and Stern-Volmer plots of  $F_0/F$  against concentration of (A) BrCl-F-Lipo (B) Lipo and (C) BrCl-F Control is marked black and consecutive spectra of the studied compounds (marked color) are in the following concentrations 1.2, 3, 6 and 9  $\mu$ M,  $\lambda_{ex} = 280$  nm,  $T = 305$  K. Spectra smoothed with SG 15 (see Method).



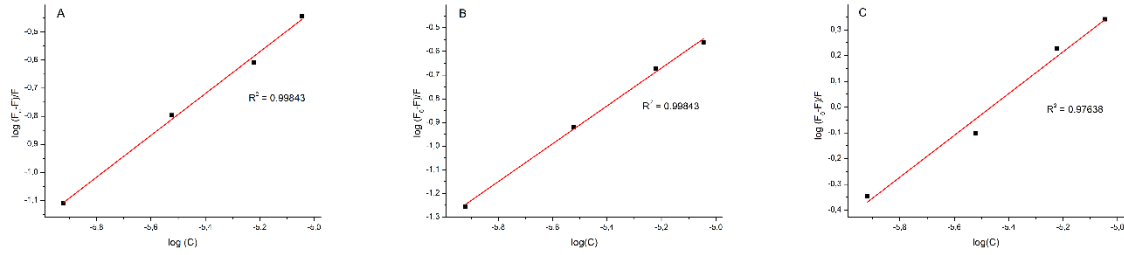
**Fig.S5** The plots of  $\log(F_0 - F)/F$  versus  $\log[c]$  of (A) BrCl-F-Lipo (B) Lipo and (C) BrCl-F at 295 K obtained from HSA fluorescence quenching.



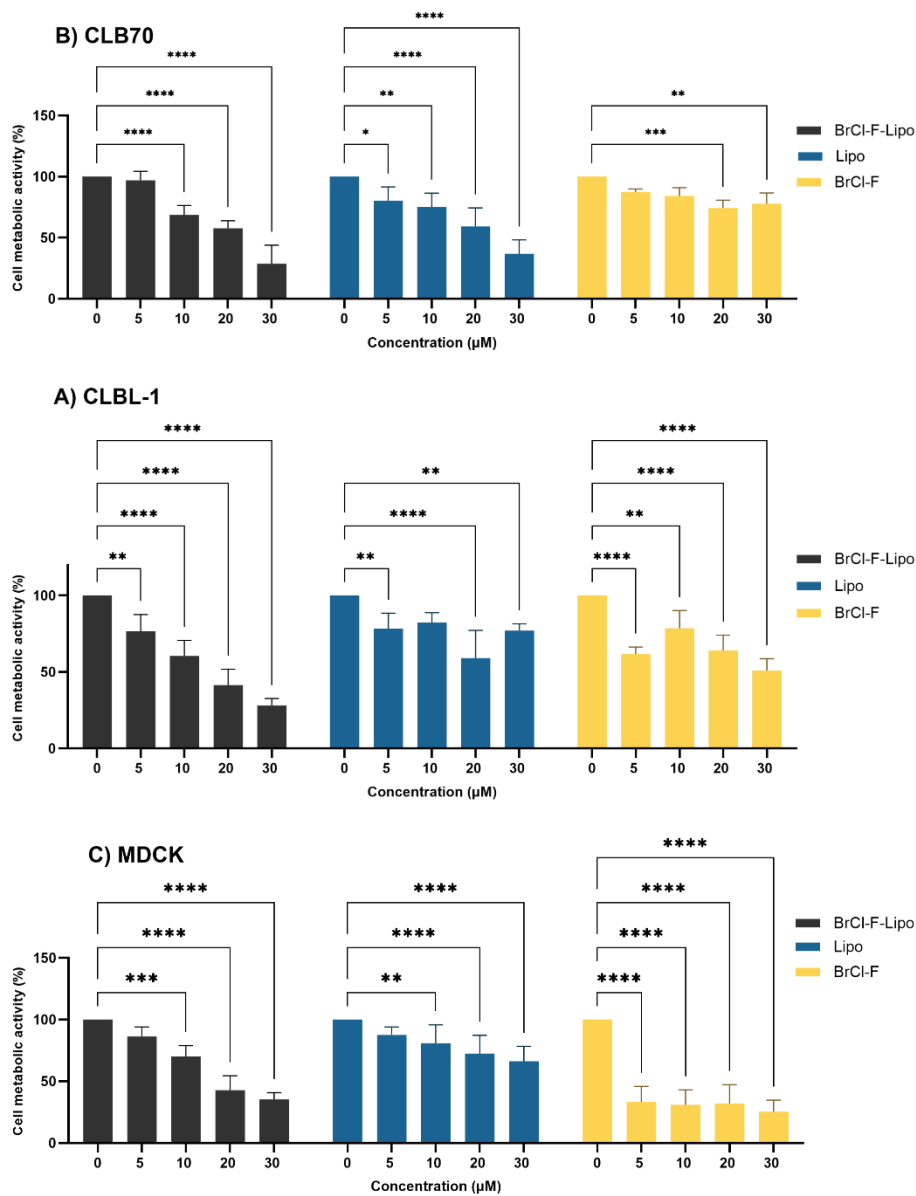
**Fig.S6** The plots of  $\log(F_0 - F)/F$  versus  $\log[c]$  of (A) BrCl-F-Lipo (B) Lipo and (C) BrCl-F at 300 K obtained from HSA fluorescence quenching.



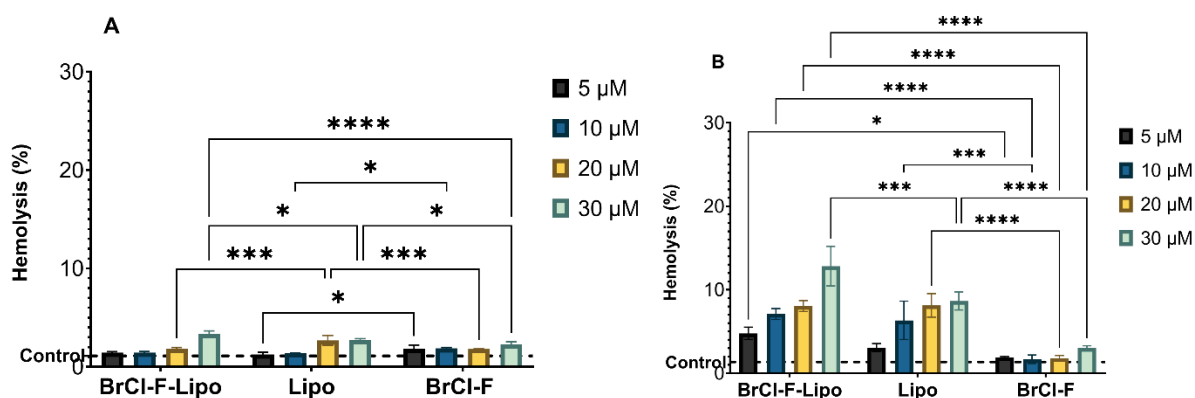
**Fig.S7** The plots of  $\log(F_0 - F)/F$  versus  $\log[c]$  of (A) BrCl-F-Lipo (B) Lipo and (C) BrCl-F at 305 K obtained from HSA fluorescence quenching.



**Fig.S8** The plots of  $\log(F_0 - F)/F$  versus  $\log[c]$  of (A) BrCl-F-Lipo (B) Lipo and (C) BrCl-F at 310 K obtained from HSA fluorescence quenching.



**Figure S9.** Effects of encapsulated BrCl-F (BrCl-F-Lipo), empty liposomes (Lipo) and 8-bromo-6-chloroflavone (BrCl-F) on metabolic activity in CLBL-1 (A), CLB70 (B) and MDCK (C) cell lines. Cells were treated with increasing concentrations (5–30  $\mu$ M) of the tested compounds for 48 h, and metabolic activity was measured by the MTT assay. Data are expressed as mean  $\pm$  SD from four independent experiments. Statistical significance vs. control: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ .



**Figure S10.** Percentage of hemolysis after 1 h (A) and 24 h (B) in the presence of the encapsulated BrCl-F (BrCl-F-Lipo), empty liposomes (Lipo) and 8-bromo-6-chloroflavone (BrCl-F). Data are expressed as mean  $\pm$  SD from three independent experiments. Statistical significance vs. control: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ .