Supplementary materials

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

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Time of storage	*Hydrodynamic diameter (nm)*	*PDI (a.u)*	*Zeta potential (mV)*
48 h	125.06 ± 1.69	0.153 ± 0.009	+28.14 ± 3.32
14 days	138.24 ± 8.36	0.16 ± 0.05	+41.8 ± 4.15
30 days	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).

Time of storage	*Hydrodynamic diameter (nm)*	*PDI (a.u)*	*Zeta potential (mV)*
48 h	141.04 ± 3.30	0.15 ± 0.02	+47.32 ± 3.13
14 days	128.1 ± 8.16	0.20 ± 0.01	+42.8 ± 4.99
30 days	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).

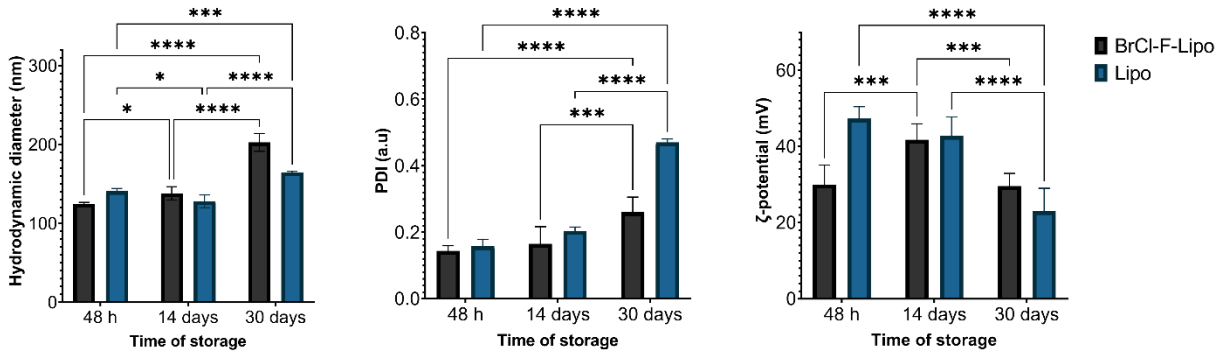

Fig.S1 Hydrodynamic diameter, polydispersity index (PDI) and zeta potential (ζ-potential) of BrCl-F-Lipo and Lipo measured after 48 h, 14 days and 30 days. Error bars represent standard deviation (n = 5). Statistical significance vs. control: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

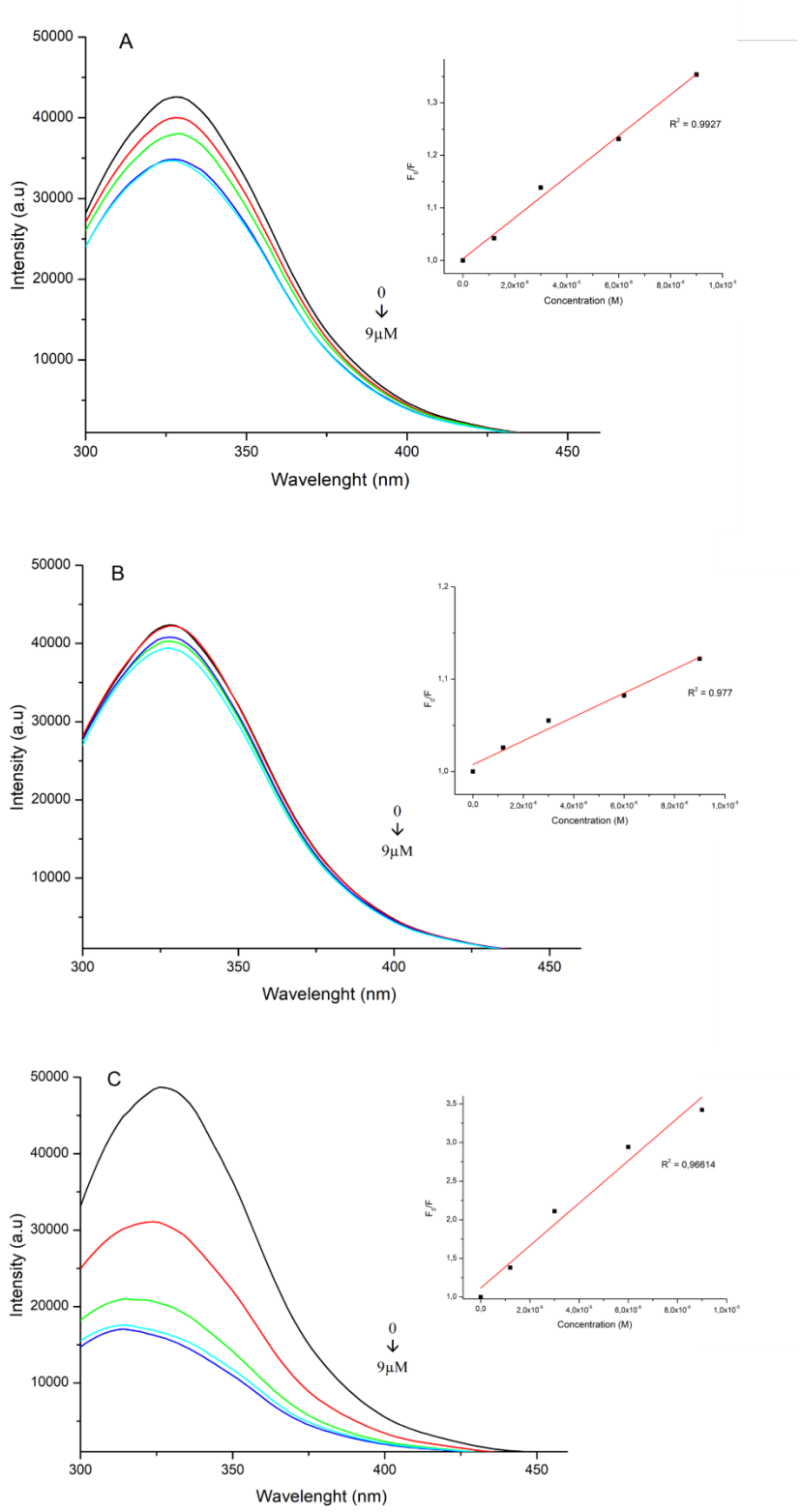

Fig. S2 Emission spectra of HSA in the presence of various concentrations and Stern-Volmer plots of F_0/F against concentration of (A) BrCl-F-Lipo (B) Lipo and (C) BrCl-F. Control is marked black and consecutive spectra of the studied compounds (marked color) are in the following concentrations 1.2, 3, 6 and 9 µM, $\lambda_{ex} = 280$ nm, T = 295 K (22 °C). Spectra smoothed with SG 15 (see Method).

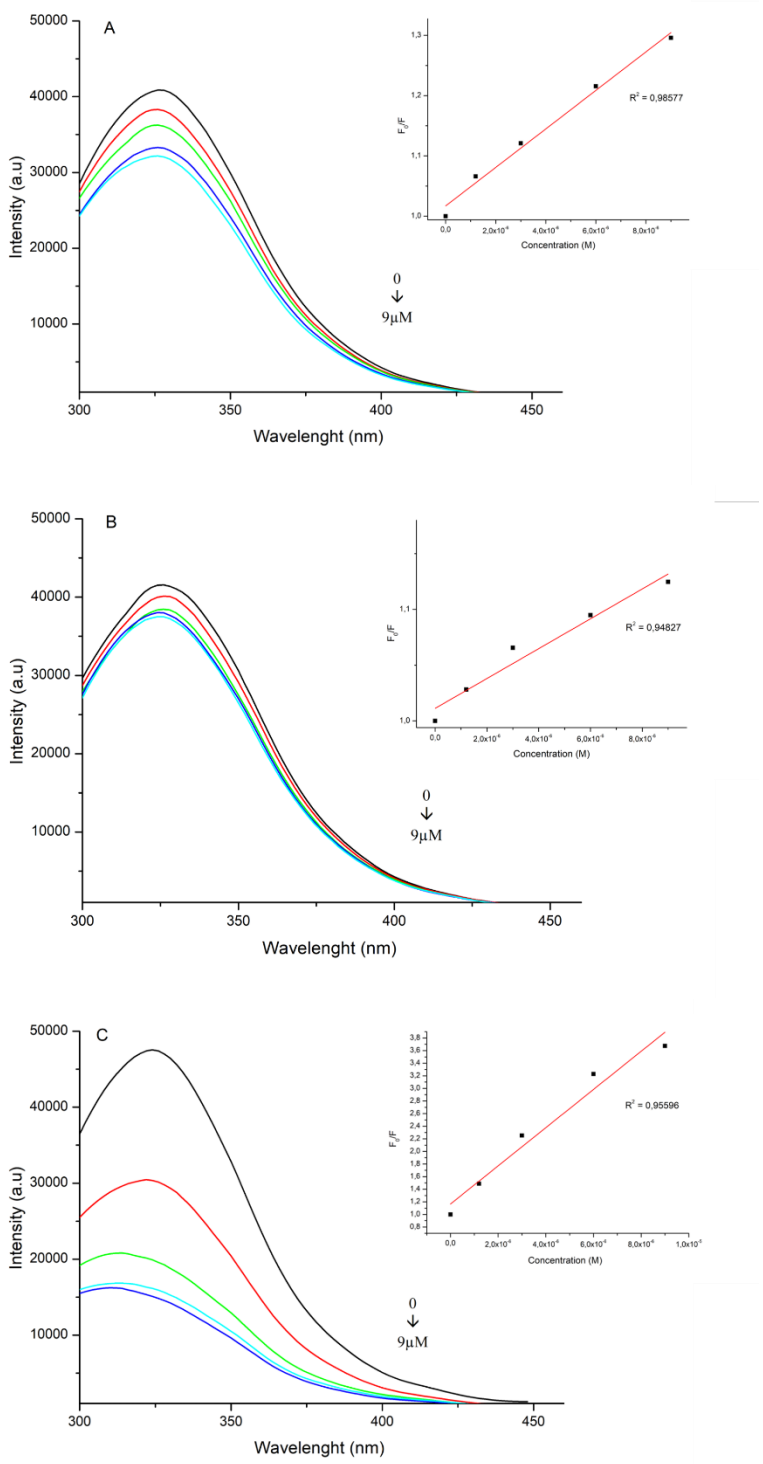

Fig. S3 Emission spectra of HSA in the presence of various concentrations and Stern-Volmer plots of F_0/F against concentration of (A) BrCl-F-Lipo (B) Lipo and (C) BrCl-F. Control is marked black and consecutive spectra of the studied compounds (marked color) are in the following concentrations 1.2, 3, 6 and 9 µM, $\lambda_{ex} = 280$ nm, T = 300 (27 °C). K. Spectra smoothed with SG 15 (see Method).

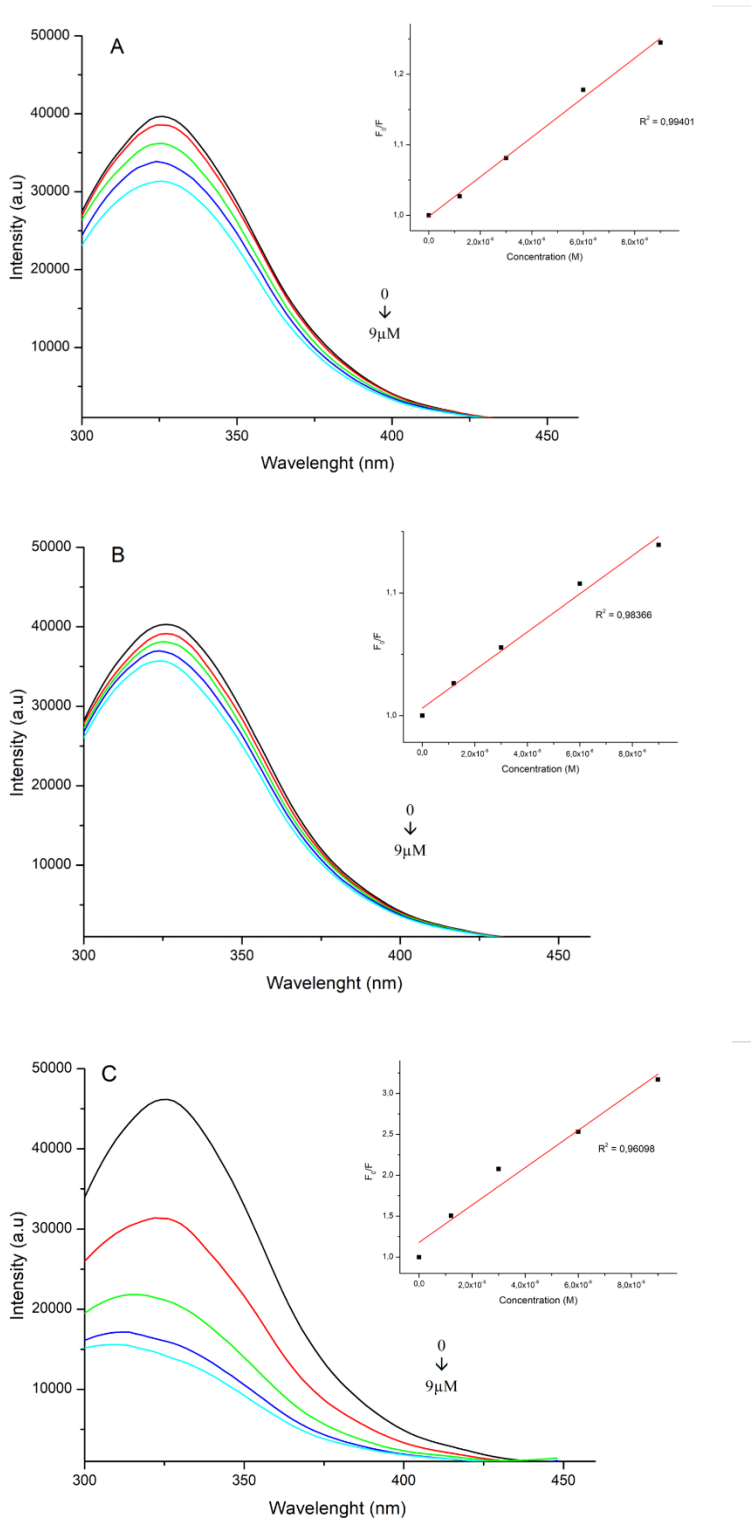

Fig. S4 Emission spectra of HSA in the presence of various concentrations and Stern-Volmer plots of F_0/F against concentration of (A) BrCl-F-Lipo (B) Lipo and (C) BrCl-F. Control is marked black and consecutive spectra of the studied compounds (marked color) are in the following concentrations 1.2, 3, 6 and 9 μM, λ_{ex} = 280 nm, T = 305 (32 °C). K. Spectra smoothed with SG 15 (see Method).

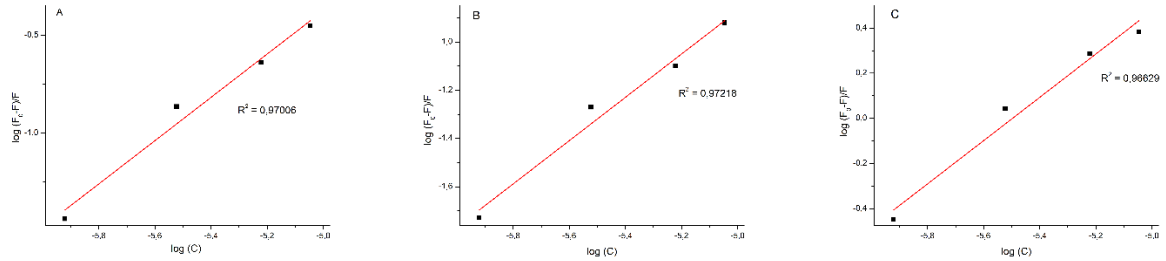

Fig.S5 The plots of log(F_0− F)/F versus log[c] of (A) BrCl-F-Lipo (B) Lipo and (C) BrCl-F at 295 K (22 ℃) obtained from HSA fluorescence quenching.

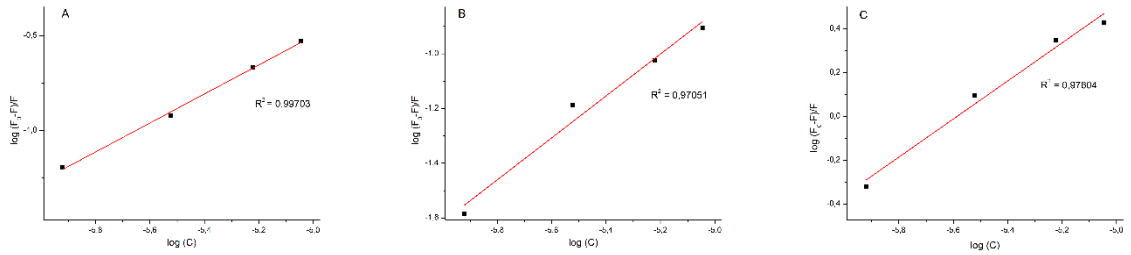

Fig.S6 The plots of log(F_0− F)/F versus log[c] of (A) BrCl-F-Lipo (B) Lipo and (C) BrCl-F at 300 K (27 ℃) obtained from HSA fluorescence quenching.

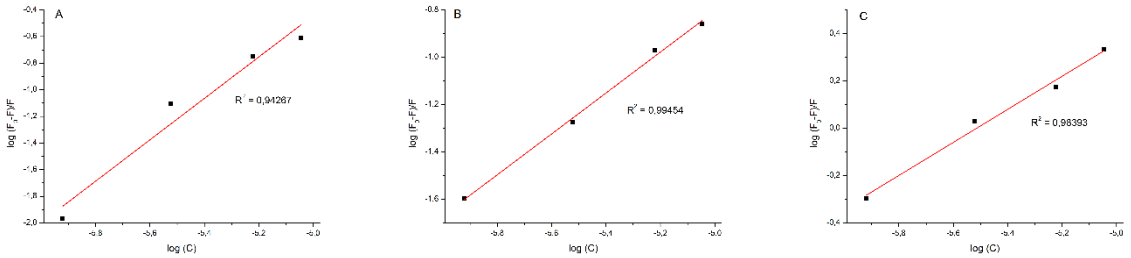

Fig.S7 The plots of log(F_0− F)/F versus log[c] of (A) BrCl-F-Lipo (B) Lipo and (C) BrCl-F at 305 K (32 ℃) obtained from HSA fluorescence quenching.

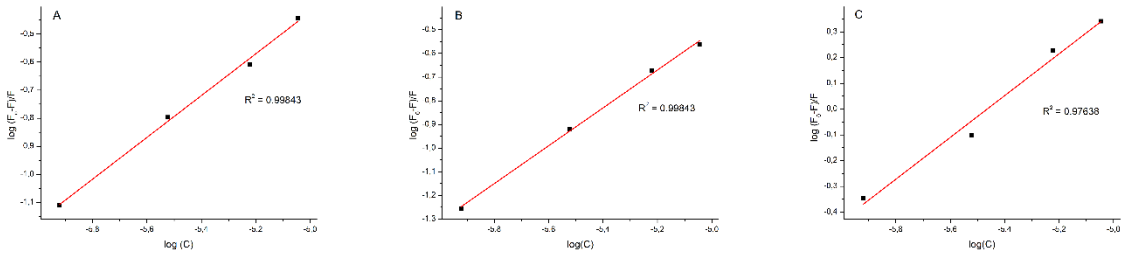

Fig.S8 The plots of log(F_0− F)/F versus log[c] of (A) BrCl-F-Lipo (B) Lipo and (C) BrCl-F at 310 K (37 °C) obtained from HSA fluorescence quenching.

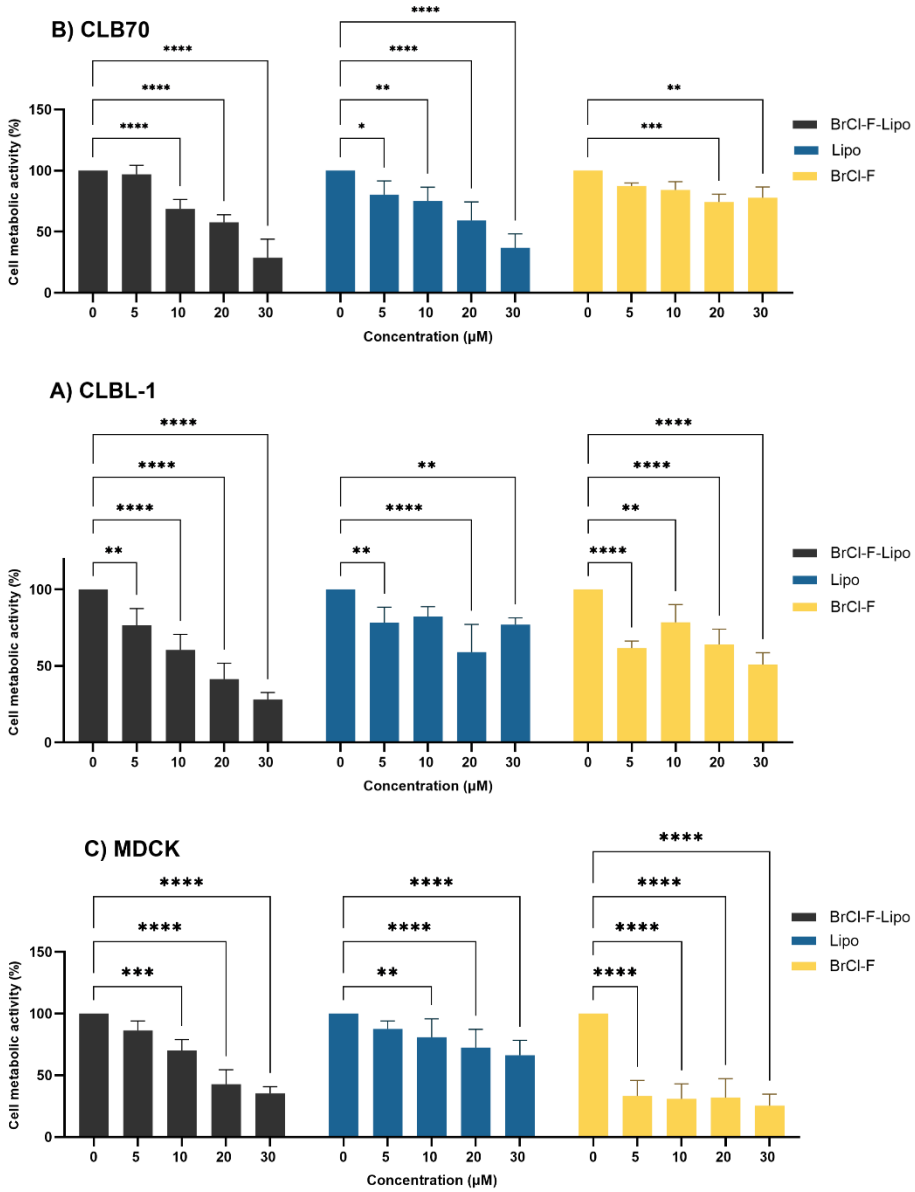

Figure S9. Effects of encapsulated BrCl-F (BrCl-F-Lipo), drug-free liposomes (Lipo) and 8-bromo-6-chloroflavone (BrCl-F) on metabolic activity in CLBL-1 (A), CLB70 (B) and MDCK (C) cell lines. Cells were treated with increasing concentrations (5–30 µM) of the tested compounds for 48 h, and metabolic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

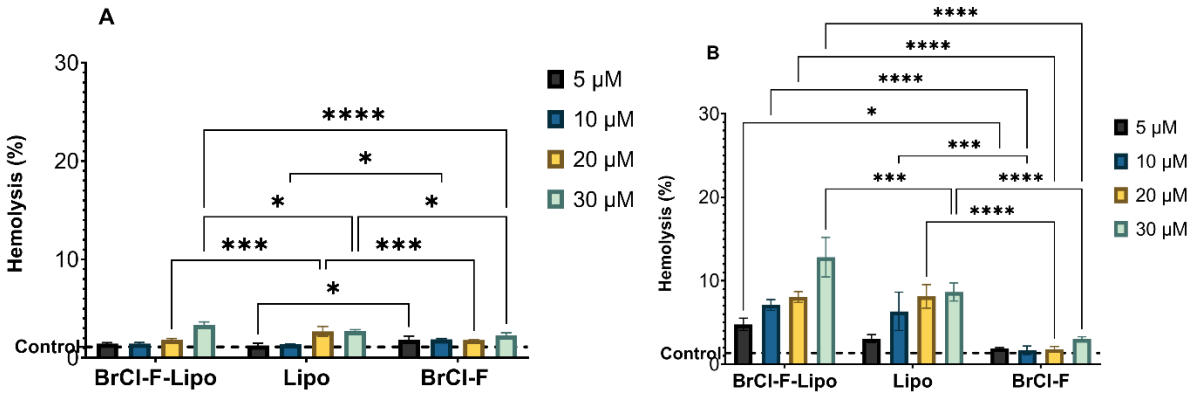

Figure S10. Percentage of hemolysis after 1 h (A) and 24 h (B) in the presence of the encapsulated BrCl-F (BrCl-F-Lipo), drug-free liposomes (Lipo) and 8-bromo-6-chloroflavone (BrCl-F). Data are expressed as mean ± SD from three independent experiments. Statistical significance vs. control: *p < 0.05;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

	Cell lines					
	MDCK		CLBL-1		CLB70	
Parameters	BrCl-F	BrCl-F-Lipo	BrCl-F	BrCl-F-Lipo	BrCl-F	BrCl-F-Lipo
LogIC$_{50}$, (95% CI)	0.6209 – 0.9475	1.173 – 1.305	1.363 – 2.056	1.142 – 1.264	1.858 – 2.834	1.221 – 1.373
Hill Slope (95% CI)	-1.230 – -0.5771	-1.678 – -1.035	-1.694 – -0.4244	-1.923 – -1.181	-0.9813 – -0.3982	-1.819 – -1.005
R^2	0.6954	0.8920	0.4990	0.9016	0.6292	0.8552
Sy.x	16.19	8.9	16.11	9.186	6.87	10.06

Tab. S3 Parameters of nonlinear regression analysis of BrCl-F and BrCl-F-Lipo concentration–response curves for MDCK, CLBL-1 and CLB70 cell lines obtained using the four-parameter logistic (variable-slope) model in GraphPad Prism. The upper and lower plateaus were constrained to 100% and 0%, respectively. Reported parameters include LogIC$_{50}$, Hill slope with 95% profile-likelihood confidence intervals (95% CI), coefficient of determination (R^2) and residual standard deviation (Sy.x).

BrCl-F concentration (µM)	**Total lipid concentration: SPC + Chol + DOTAP (µM)**	**DOTAP concentration (µM)**	**TPGS concentration (µM)**
5	159.9	2.52	8.42
10	319.8	5.05	16.83
20	639.6	10.10	33.66
30	959.4	15.15	50.49

Tab. S4 Final concentrations of total lipid, DOTAP, and TPGS corresponding to the nominal BrCl-F concentrations used in the cell-based experiments. Total lipid was defined as the combined concentration of SPC, cholesterol, and DOTAP, while TPGS was reported separately. Total lipid, DOTAP, and TPGS concentrations were calculated based on the BrCl-F concentration recovered in the filter-passing formulation (i.e., corrected for encapsulation efficiency), rather than on the nominal drug input used during liposome preparation.