Supporting Information

Hyaluronic Acid Layered Chimeric Nanoparticle: Targeting MAPK-PI3K Signaling Hub in Colon Cancer Cells

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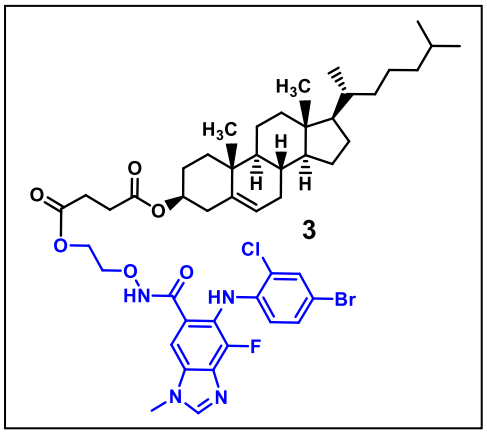
Content:

Characterization of cholesterol-AZD6244 conjugate (3):



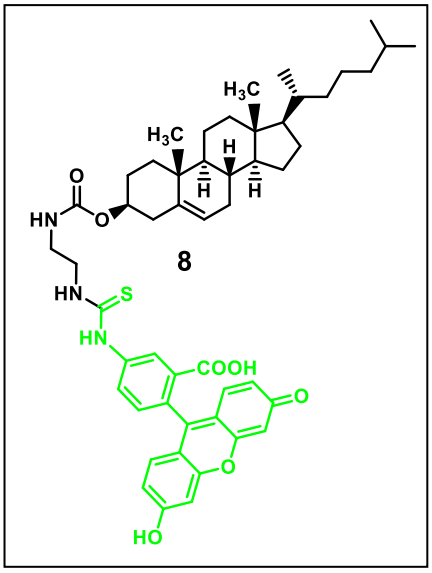
^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.91 (s, 1H), 8.23 (s, 1H), 8.00 (s, 1H), 8.05 (s, 1H), 7.49 (d, *J* = 2.2 Hz, 1H), 7.16-7.18 (dd, *J* = 8.8, 2.2 Hz, 1H), 6.55 – 6.52 (m, 1H), 5.35 (s, 1H), 4.59 (s, 2H), 4.23 (m, 2H), 3.93 (s, 3H), 2.66 (s, 2H), 2.39 (s, 1H), 2.29 (d, *J* = 7.6 Hz, 2H), 2.01– 1.8 (m, 4H), 1.57 – 1.41 (m, 14H), 1.25 (m, 3H), 1.11 (m, 7H), 0.99 (s, 4H), 0.85 – 0.92 (m, 10H), 0.67 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.34, 147.37, 139.70, 139.62, 131.69, 130.23, 124.96, 122.97, 122.46, 117.99, 116.26, 116.22, 111.29, 109.52, 77.16, 74.88, 73.98, 62.37, 59.67, 56.83, 56.27, 50.15, 42.46, 39.86, 39.66, 38.30, 38.20, 37.07, 36.72, 36.33, 35.94, 32.04, 31.98, 31.78, 31.39, 29.85, 29.64, 29.61, 28.37, 28.17, 27.87, 24.42, 23.97, 22.97, 22.71, 21.16, 19.43, 18.86, 11.57.

^{19}F NMR (376 MHz, CDCl3): δ = -129.16

MALDI-TOF (m/z): Calculated mass = 924.3604 for $C_{48}H_{63}BrClFN_4O_6$, observed = 925.4789 for $C_{48}H_{64}BrClFN_4O_6$ $[M+H]^+$.

Characterization of FITC-Cholesterol conjugate (8):



^{1}H NMR (400 MHz, MeOD): δ = 7.80 (s, 1H), 7.58 (d, *J* = 7.7 Hz, 1H), 7.16 – 7.23 (m, *J* = 16.4, 8.8 Hz, 3H), 6.57 (s, 1H), 6.55 (s, 3H), 5.22 (s, 1H), 3.81 – 3.68 (m, 2H), 3.46 – 3.35 (m, *J* = 6.5 Hz, 2H), 2.23 (s, 1H), 2.02 (s, 1H), 1.96 – 1.77 (m, 4H), 1.78 – 1.64 (m, 2H), 1.63 – 1.47 (m, *J* = 6.9 Hz, 3H), 1.47 – 1.33 (m, 8H), 1.33 – 1.24 (m, 3H), 1.22 – 1.08 (m, 5H), 0.99 – 0.84 (m, 14H), 0.68 (s, 3H).

^{13}C NMR (101 MHz, MeOD): δ = 182.81, 180.80, 173.47, 160.16, 160.11, 141.44, 132.93, 132.80, 126.46, 126.36, 123.67, 123.52, 123.49, 114.46, 114.37, 104.60, 104.51, 100.95, 75.74, 58.59, 58.19, 57.83, 51.71, 49.00, 47.21, 45.12, 43.69, 41.31, 40.90, 39.95, 38.31, 37.76, 37.60, 37.37, 35.52, 33.99, 33.31, 33.02, 29.53, 29.35, 25.49, 25.24, 24.36, 23.40, 23.15, 22.30, 19.93, 19.48, 12.50.

MALDI-TOF (m/z): Calculated mass = 861.4387 for $C_{51}H_{63}N_3O_7S$, observed = 862.73 for $C_{51}H_{64}N_3O_7S$ $[M+H]^+$.

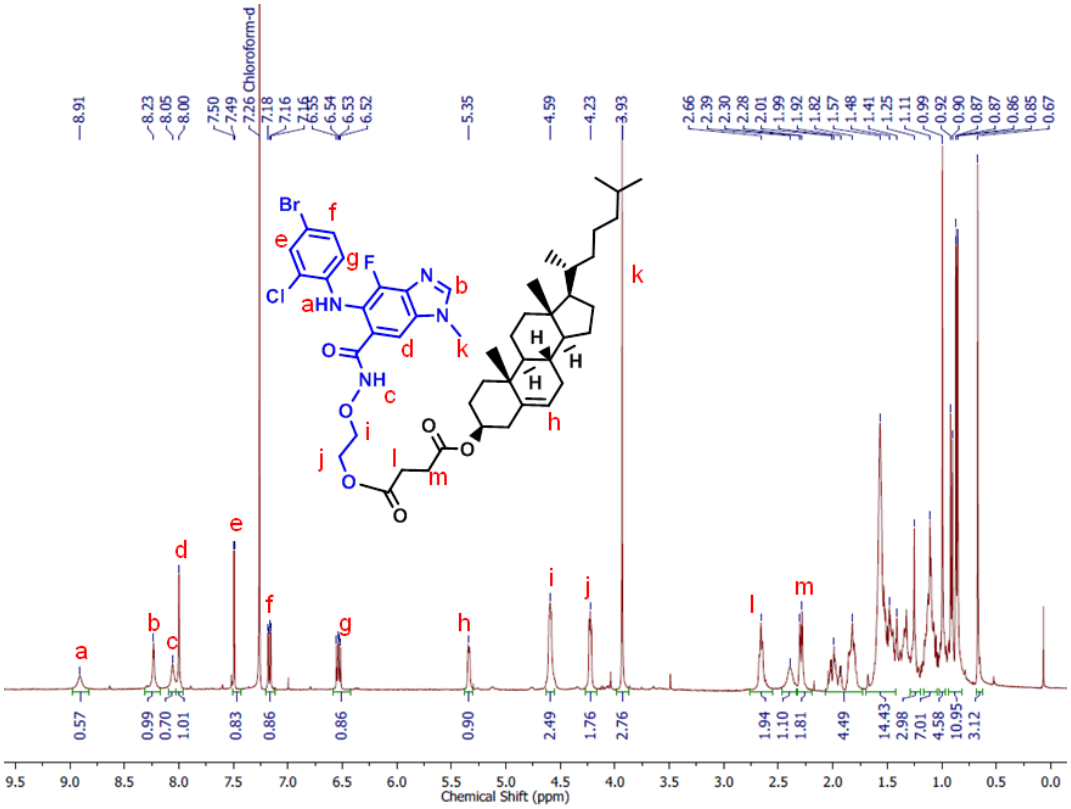


Figure S1: ^{1}H NMR spectra of cholesterol-AZD6244 conjugate (3).

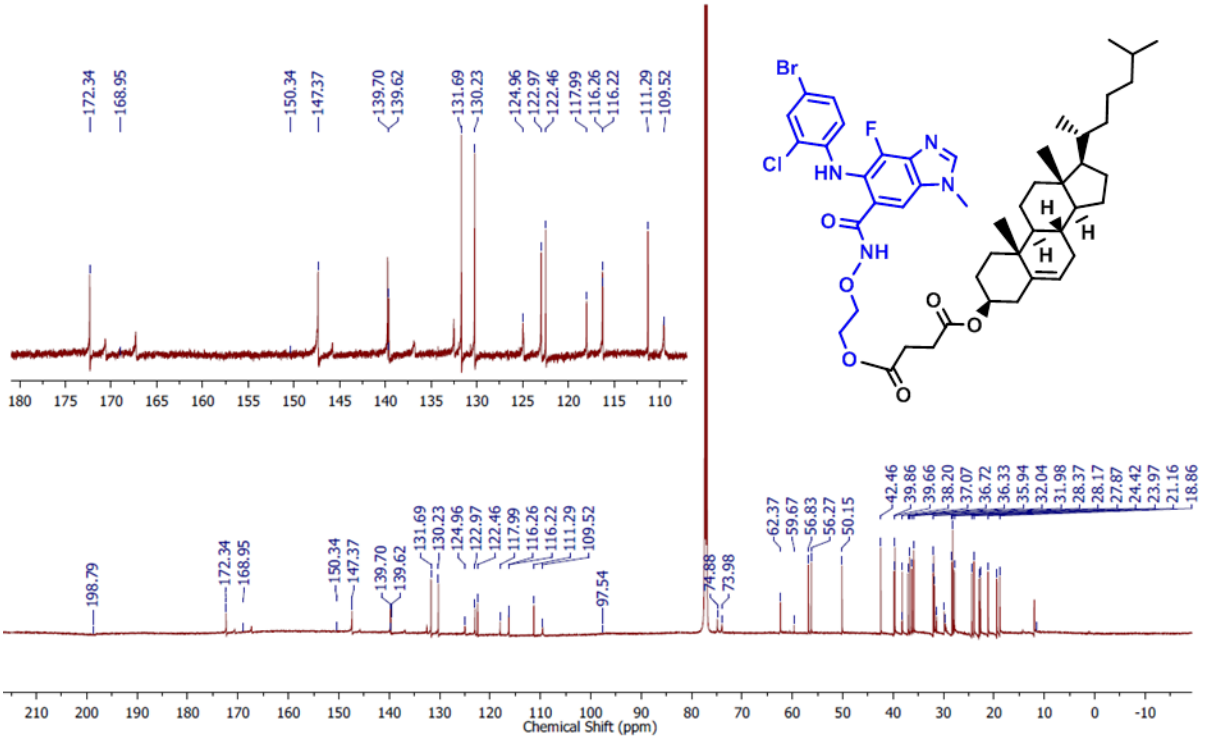


Figure S2: ^{13}C NMR spectra of cholesterol-AZD6244 conjugate (3).

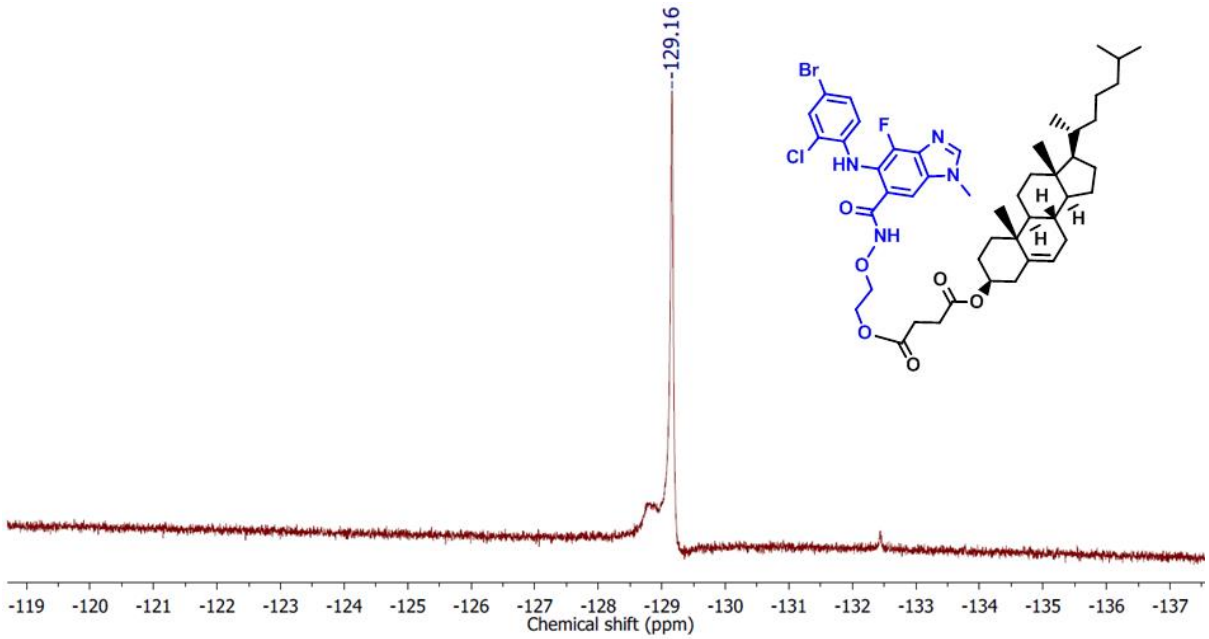


Figure S3: ^{19}F NMR spectra of cholesterol-AZD6244 conjugate (3).

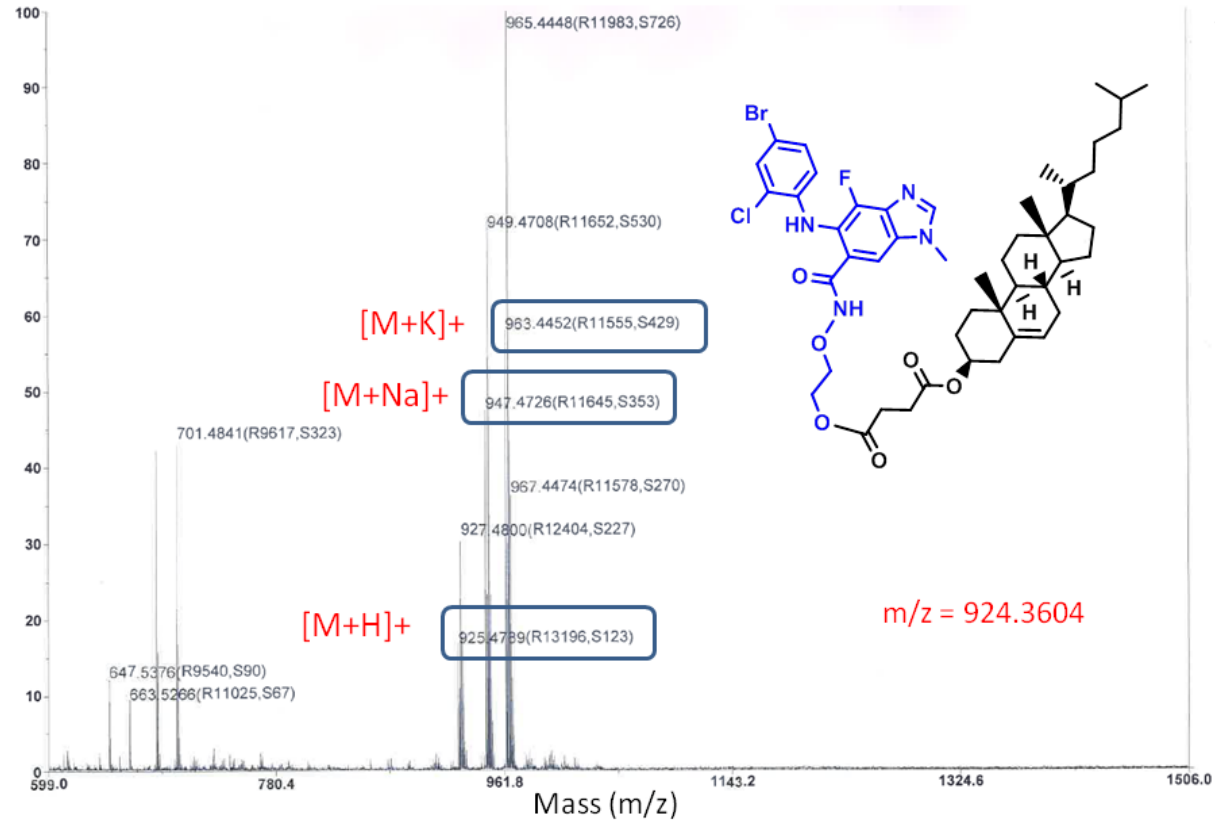


Figure S4: MALDI-TOF spectra of cholesterol-AZD6244 conjugate (3).

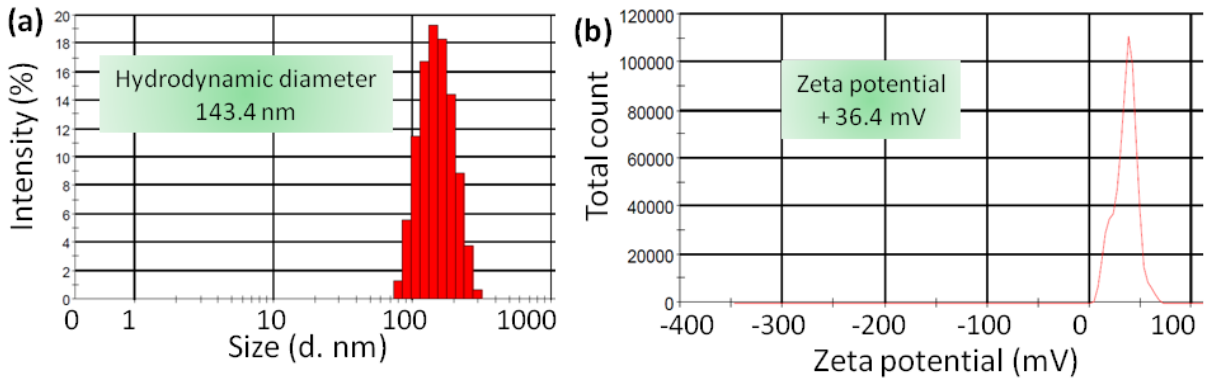


Figure S5: (a) Hydrodynamic diameter and (b) zeta potential of chimeric nanoparticle (CNP) determined by dynamic light scattering (DLS).

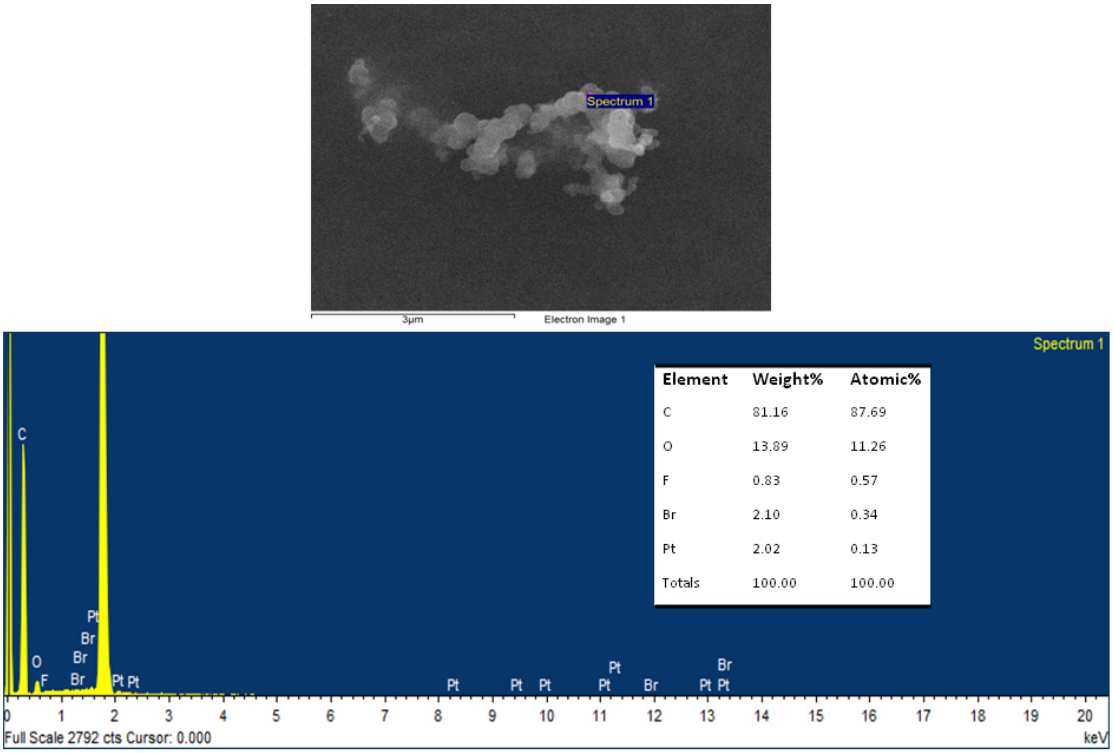


Figure S6: Energy-dispersive X-ray spectroscopy (EDX) of HA-CNPs to validate the presence of AZD6244 and cisplatin in the same particle.

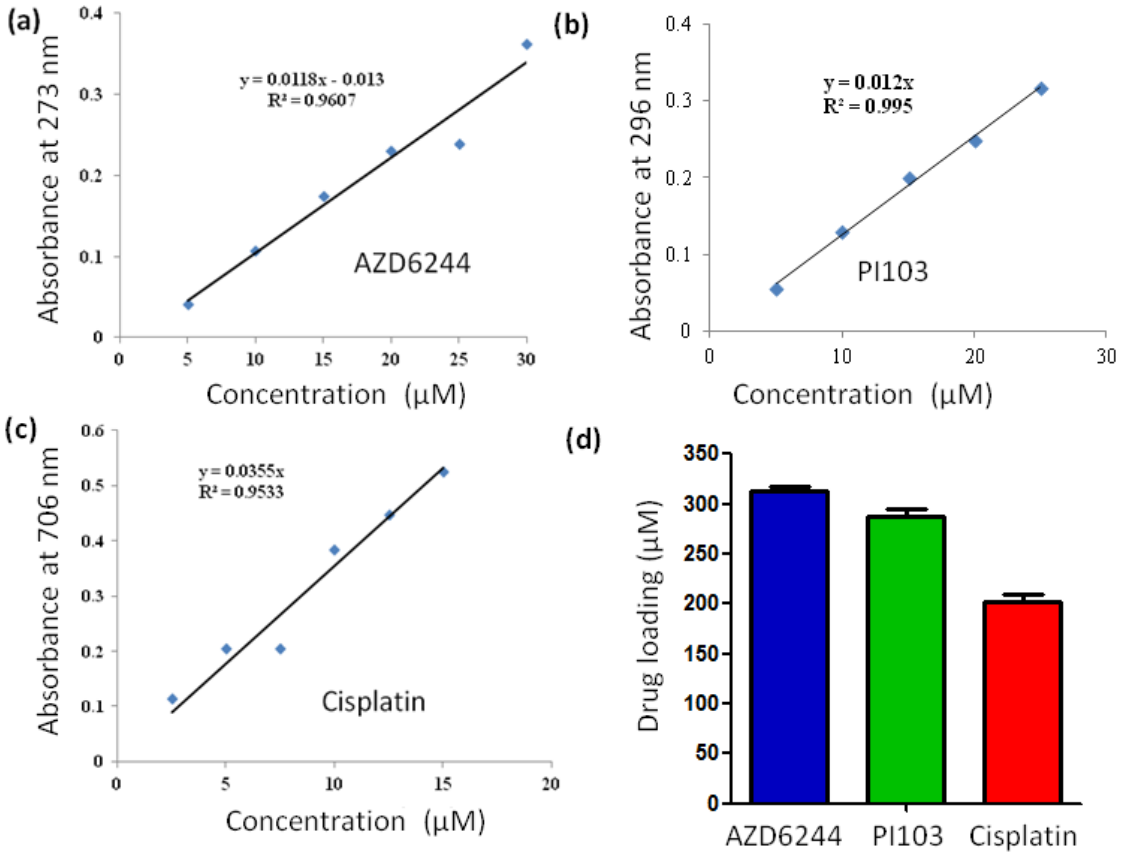


Figure S7: (a-c) Concentration versus absorbance calibration graph of AZD6244, PI103 and cisplatin at λ_{max} = 273 nm, 296 nm and 706 nm respectively from UV-Vis spectroscopy. (d) Loading of AZD6244, PI103 and cisplatin in HA-CNPs determined by UV-Vis spectroscopy from concentration versus absorbance calibration graph.

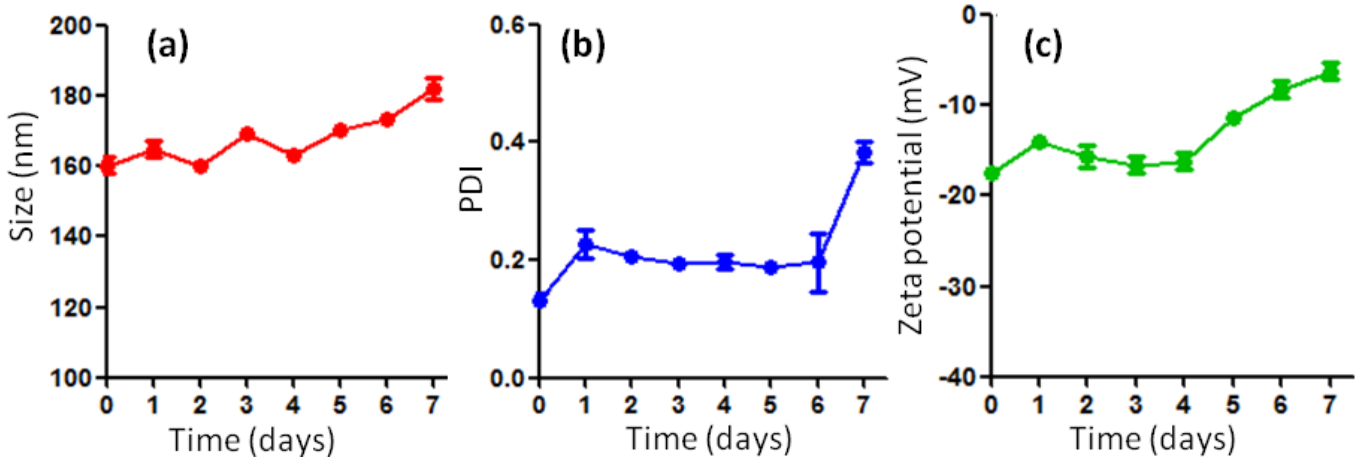


Figure S8: Stability of HA-CNPs was evaluated through DLS by measuring (a) hydrodynamic diameter (b) poly-dispersity index (PDI) and (c) surface charge in DMEM media containing 10 % FBS at 37 °C over 7 days.

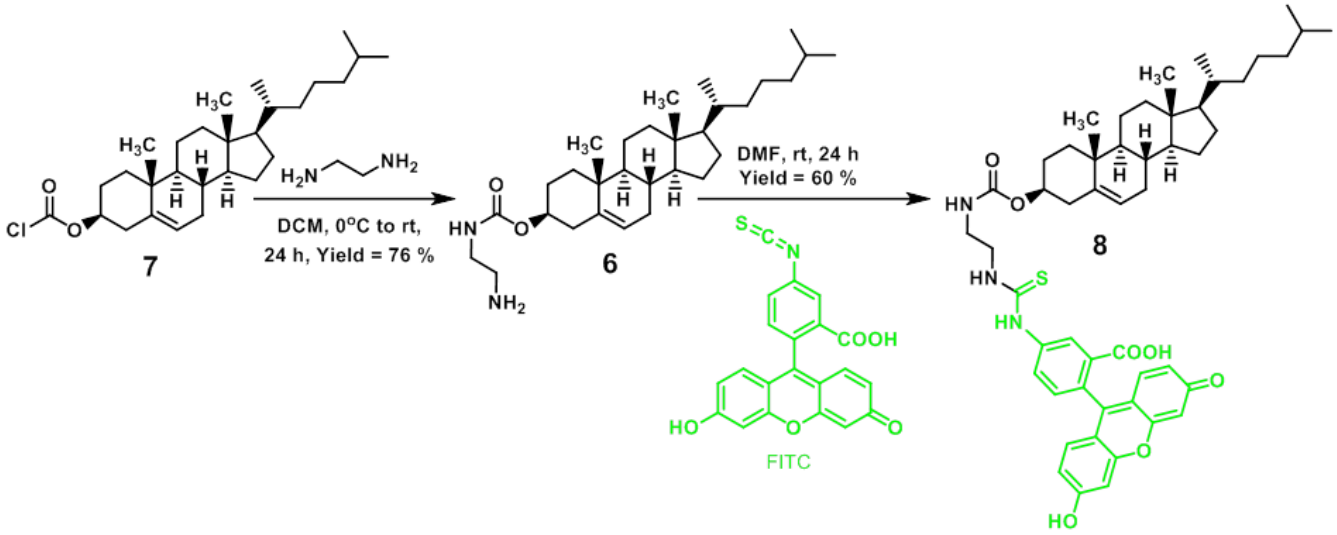


Figure S9: Synthetic scheme of FITC-cholesterol conjugate (8).

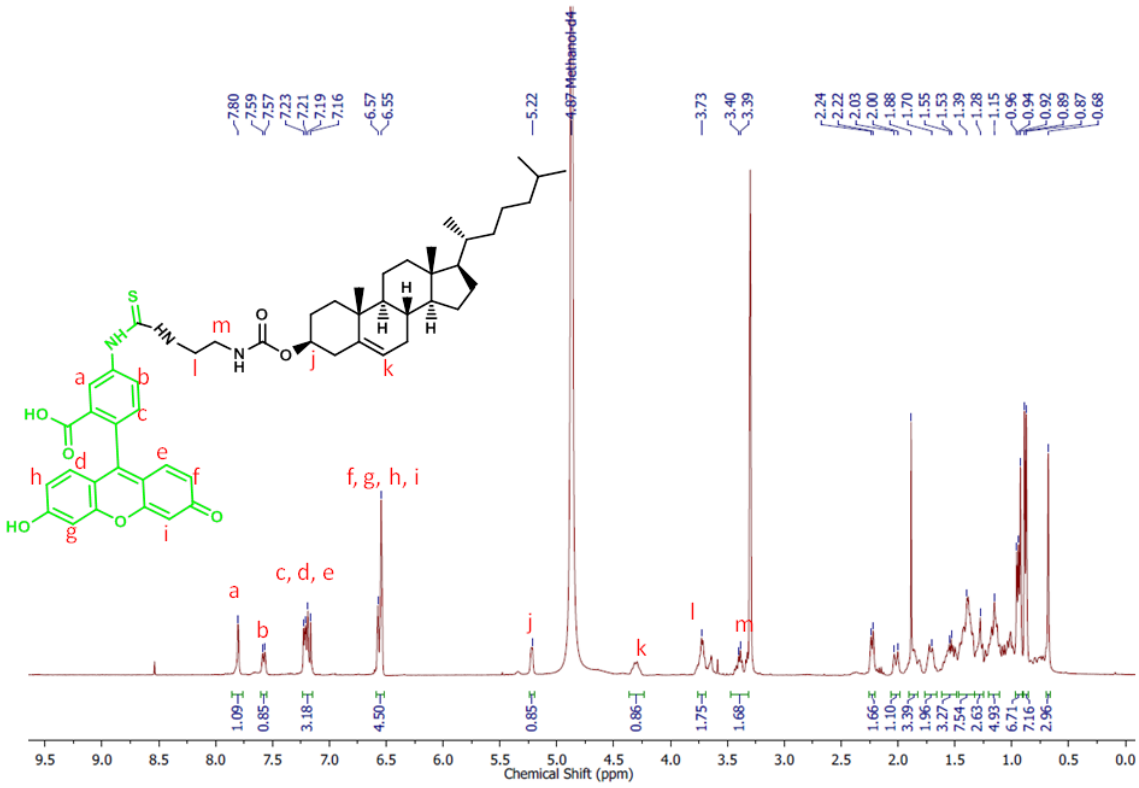


Figure S10: ^{1}H NMR spectra of FITC-cholesterol conjugate (8).

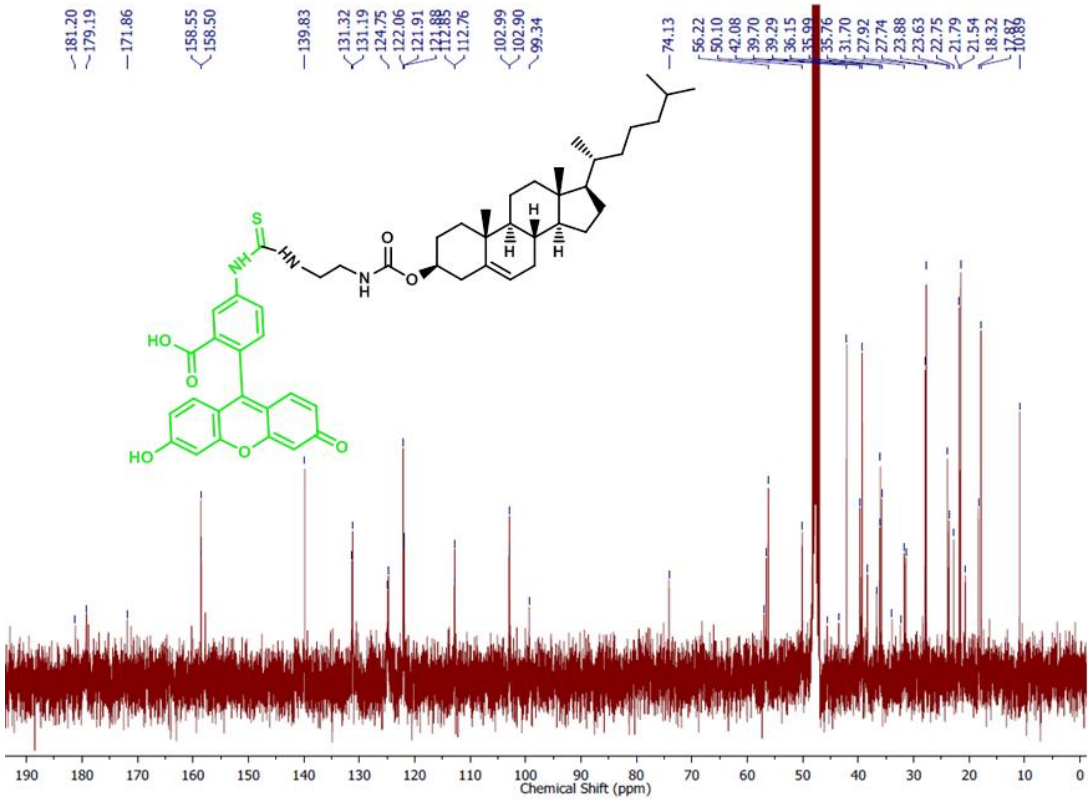


Figure S11: ^{13}C NMR spectra of FITC-cholesterol conjugate (8).

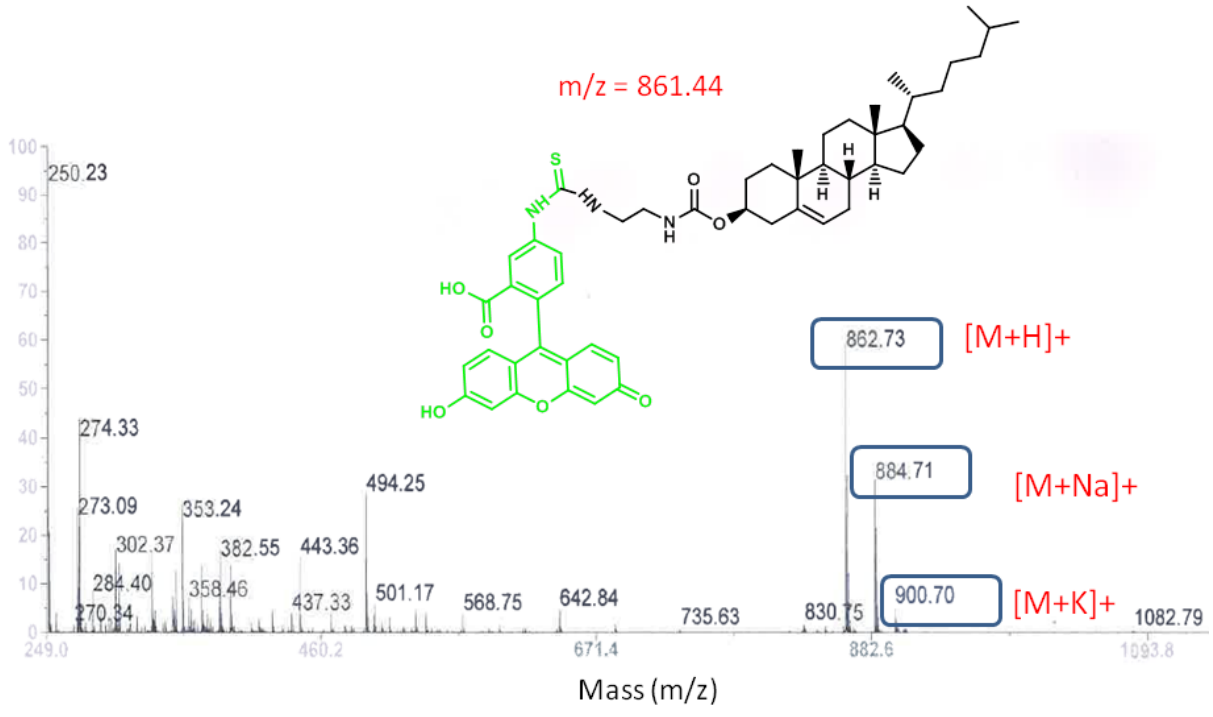


Figure S12: MALDI-TOF spectra of FITC-cholesterol conjugate (8).

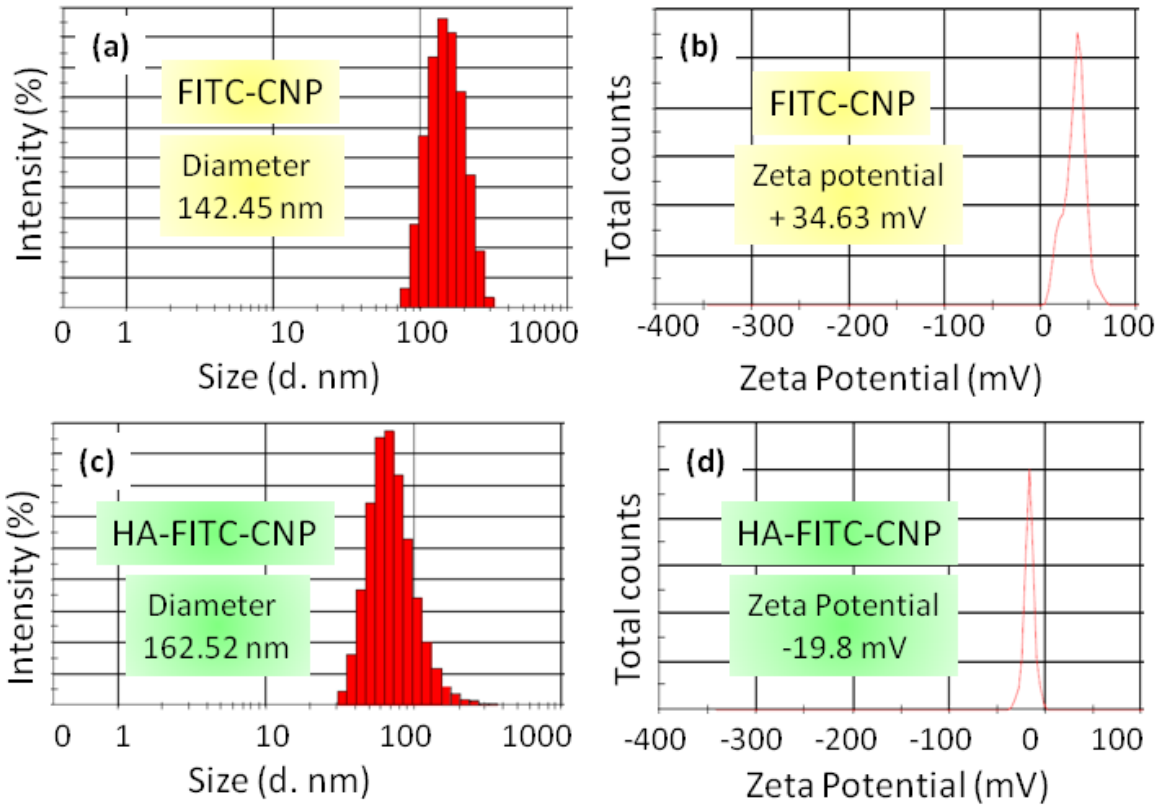


Figure S13: (a, c) Hydrodynamic diameter of FITC-CNP and HA-FITC-CNP; (b,d) zeta potential of FITC-CNP and HA-FITC-CNP determined by DLS measurements.

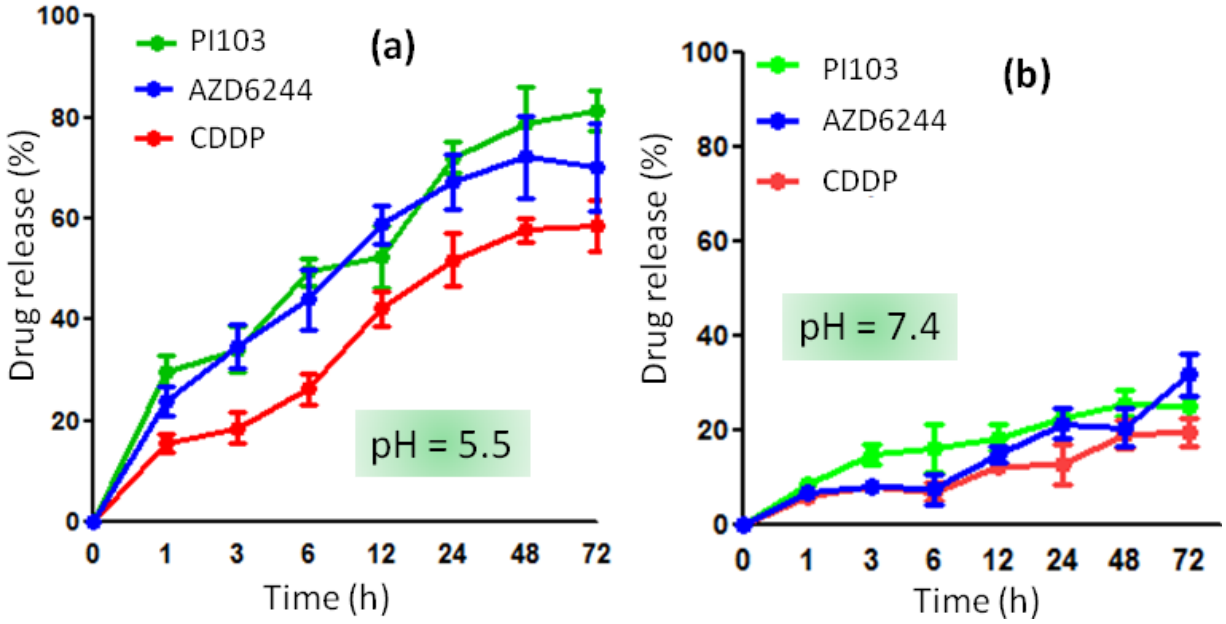


Figure S14: Time dependent release of AZD6244, PI103 and cisplatin from HA-CNP at (a) pH = 5.5 and (b) pH = 7.4.

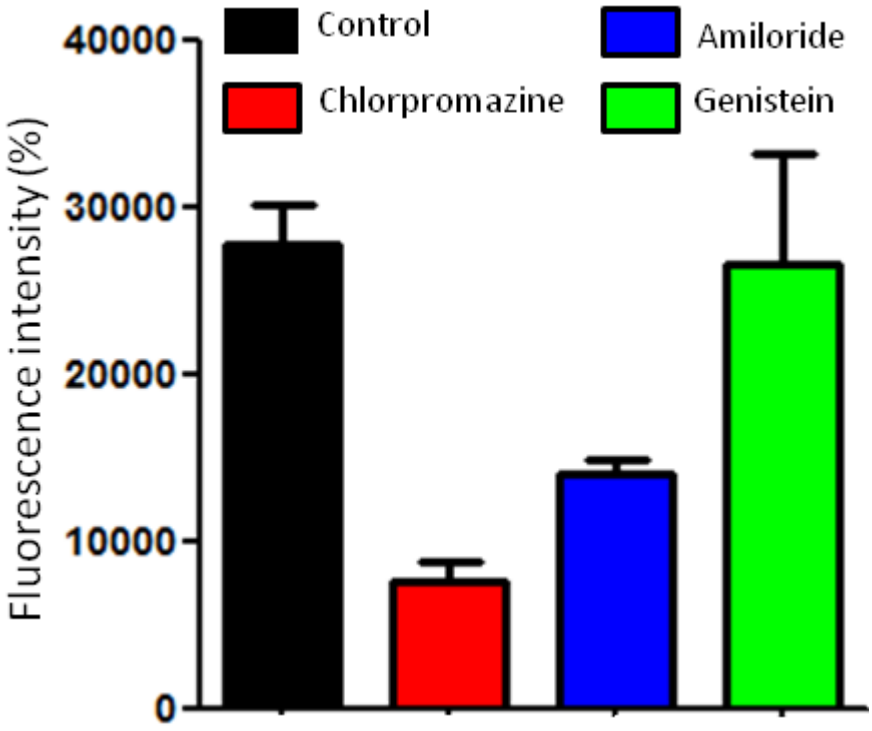


Figure S15: Quantification of green fluorescence intensity in HCT-116 cells after treatment with endocytosis inhibitors followed by HA-FITC-CNPs, determined from CLSM.

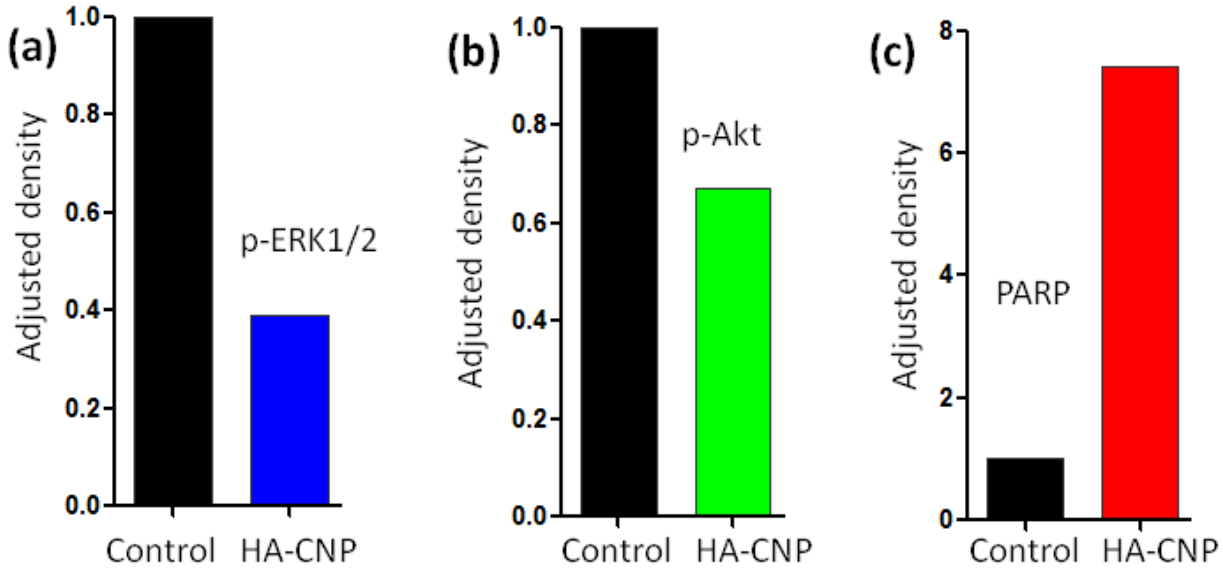


Figure S16: Quantification of the expression of (a) *p*-ERK1/2, (b) *p*-Akt and (c) PARP from Western blot analysis in HCT-116 cells after treatment with HA-CNPs for 24 h.

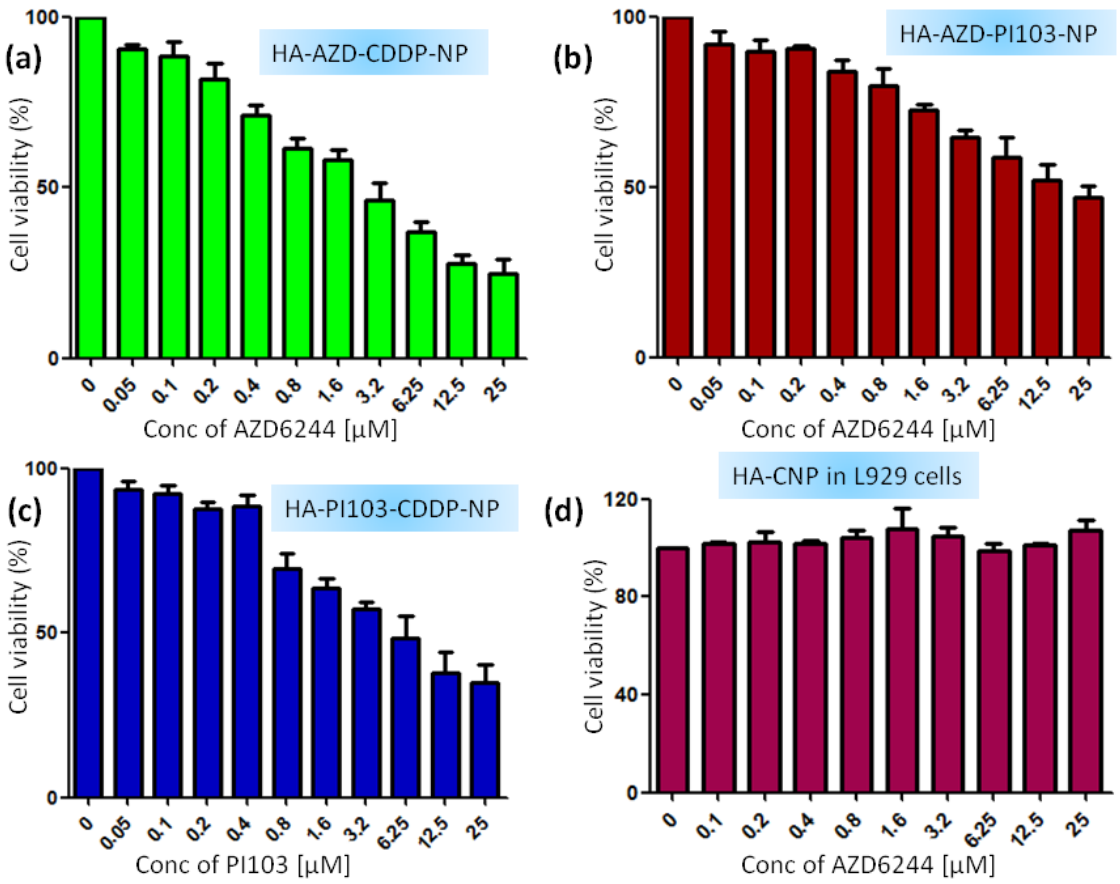


Figure S17: Concentration dependent cell viability of (a) HA-AZD-CDDP-NPs, (b) HA-AZD-PI103-NPs, (c) HA-PI103-CDDP-NPs in HCT-116 cells after 24 h determined by MTT assay. (d) Concentration dependent cell viability of HA-CNPs in L929 fibroblast cells at 24 h post treatment determined by MTT assay.

Treatment Time (h)	**Image Channels**			
	C1 (red), C2 (green)			
	Pearson's Correlation Coefficient	Manders Coefficients		**Percent volume co-localization**
		M1 (fraction of C1 over-lapping C2)	M2 (fraction of C2 over-lapping C1)	
1	0.9741	0.8997	0.9126	77.3
3	0.9476	0.6499	0.6676	40.62
6	0.978	0.7521	0.7966	23.44

Table S1: Quantification of homing of FITC-HA-CNPs into lysosomes stained with LysoTracker Red from CLSM.