pH-sensitive liposome formulation of peptidomimetic-doxorubicin conjugate for targeted delivery of anticancer conjugate on HER2 positive lung and breast cancer

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Background

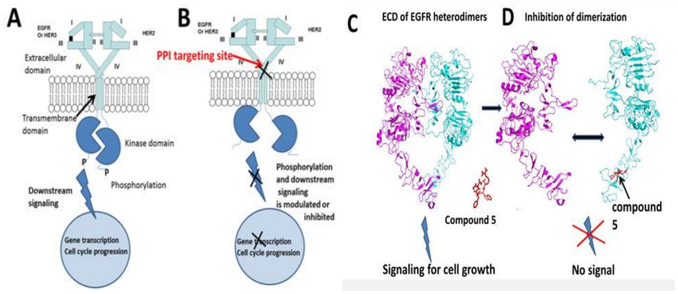

Fig 1: Importance of EGFR in cell signaling. A) Dimerization of extracellular domains by overexpression or ligand binding results in phosphorylation of kinase domain and downstream signaling for cell growth.

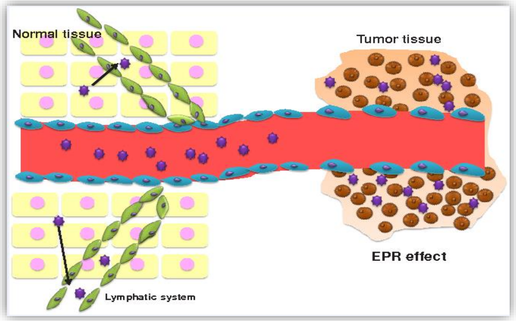

Fig 2: The enhanced permeability and retention (EPR) effect

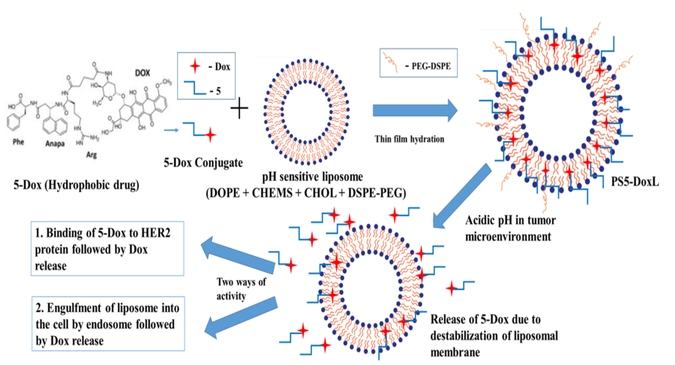

Fig. 3 The schematic diagram for preparation and activity of PS5-DoxL.

Formulation: (PSC5-DOXL)DOPE : CHEMS : CHOL : DSPE-PEG 2000 : 5-Dox – 4: 2.5 : 2 : 0.5 : 1 molar ratio
Lipid : Drug ratio – 9 : 1

Results

Table 1 The characterization of PS5-DoxL, PS-DoxL and, NPS5-DoxL (n=3)

Liposomes	Diameter (nm)	PDI	Zeta-potential (mV)	Entrapment Efficiency
PS5-DoxL	170.34 ± 3.75	0.209 ± 0.016	- 24.57 ± 4.68	88.45 ± 1.50
PS-DoxL	155.57 ± 3.62	0.220 ± 0.013	-6.91 ± 1.23	88.94 ± 0.48
NPS5-DoxL	155.52 ± 4.01	0.281 ± 0.009	- 15.73 ± 0.50	92.32 ± 1.98
Plain PSL	135.33 ± 1.78	0.169 ± 0.019	-28.81 ± 1.59	-

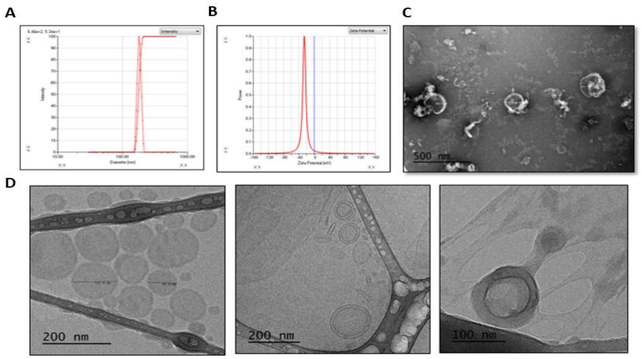

Fig 4. (A) Particle Size distribution and (B) Zeta Potential graphs of PS5-DoxL (C) TEM and (D) cryo-TEM images are showing the morphological characteristics of PS5-DoxL at different magnifications.

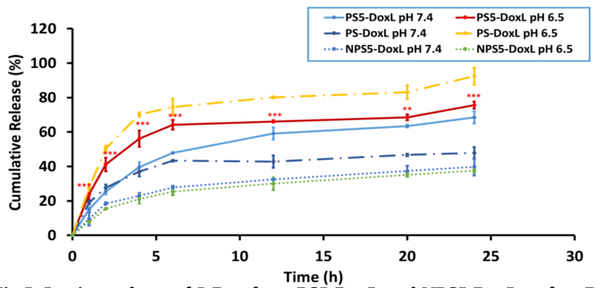

Fig 5. In vitro release of 5-Dox from PS5-DoxL and NPS5-DoxL or free Dox from PS-DoxL at 37 °C, pH 6.5 and 7.4 PBS buffer medium, respectively. Data represent the mean ± SD (n=3). Statistical significance between PS5-DoxL 7.4 and PS5-DoxL 6.5 (p <0 .01 and *** p <0 .001).**

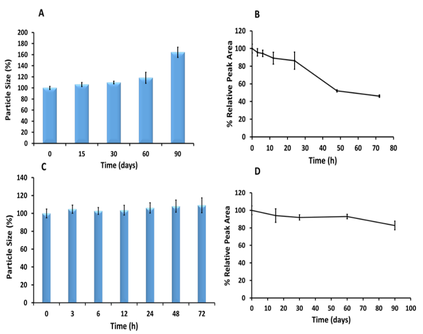

Fig 6. Stability data of (A) PS5-DoxL long term stability stored at 4° C up to 90 days analyzed in particle size analyzer, (B) 5-Dox in PS5-DoxL stored at 4° C up to 90 days analyzed in HPLC, (C) PS5-DoxL in human serum up to 72 h, and D. 5-Dox in PS5-DoxL in human serum up to 72 h analyzed in HPLC.

Table 2 Antiproliferative activity of PS5-DoxL, 5-Dox, and Free Dox in Calu-3, A549, BT474, MCF-7, and HLFs

	IC_{50} in µM (72 Hours)				
Compound	Calu-3	A549	BT474	MCF-7	HLFs
PS5-DoxL	0.540 ± 0.029	0.780 ± 0.032	0.617 ± 0.168	5.424 ± 1.957	> 50
5-Dox	0.532 ± 0.082	0.484 ± 0.138	0.633 ± 0.147	3.841 ± 0.205	5.447 ± 0.081
Free Dox	0.158 ± 0.075	0.490 ± 0.079	0.399 ± 0.079	0.316 ± 0.165	0.170 ± 0.082
Plain PSL	> 50	> 50	> 50	> 50	> 50

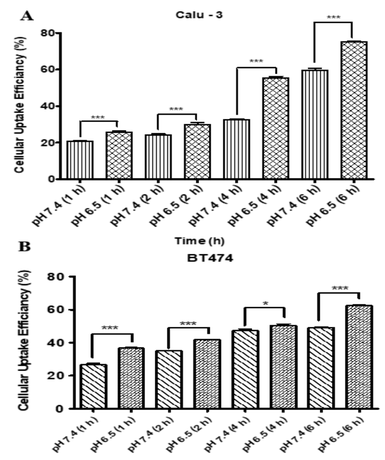

Fig 7. In vitro cellular uptake analysis by spectrofluorometer up to 6h in (A) Calu-3 and (B) BT474 cell lines treated with PS5-DoxL in pH 7.4 and pH 6.5.Notes: Data represent the mean ± SD (n=3). Statistical significance between pH 7.4 and pH 6.5 (* p <0.05, ** p <0.01, and *** p <0 .001).

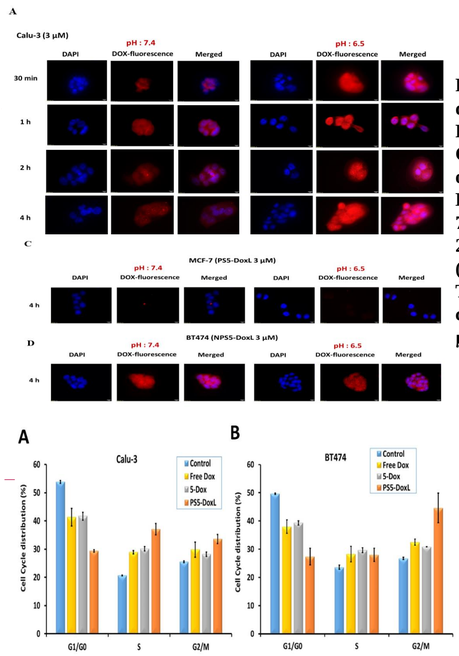

Fig 8. Time-dependent uptake of PS5-doxL in (A) Calu-3 (B) Uptake of NPS5-DoxL in BT474 cells in pH 7.4 and pH 6.5 for 2h. (60x magnification). The scale bar corresponds to 10 µm.

Fig. 9 Effect of 5-Dox and PS5-DoxL on cell cycle arrest in HER2-overexpressed cancer cells (A) Calu-3 and (B) BT474. Data represented as mean ± standard deviation.

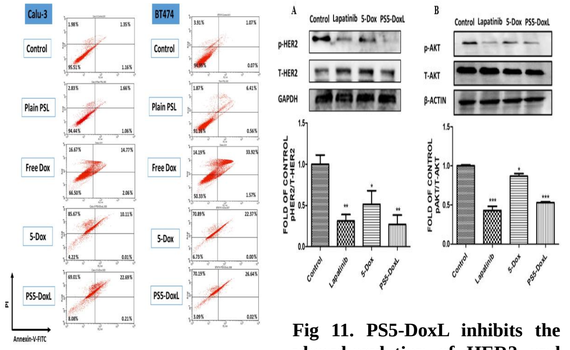

Fig. 10 Apoptosis study on Calu-3 and BT474 after treatment with 5-Dox, Free Dox, and PS5-DoxL for 12 h.

Fig 11. PS5-DoxL inhibits the phosphorylation of HER2 and Akt.
Data represented as mean ± standard deviation *p<0.05, **<0.01, and *** P<0.001.

Table 3
Antiproliferative activity in multicellular 3D tumor spheroids of Calu-3, BT474, and MCF-7 treated with PS5-DoxL, 5-Dox, and Free Dox.

	3D tumor spheroid IC_{50} in µM (72 Hours)		
Compound	Calu-3	BT474	MCF-7
PS5-DoxL	0.660 ± 0.140*#	0.728 ± 0.123*	3.602 ± 0.137
5-Dox	0.687 ± 0.148	0.755 ± 0.024	5.157 ± 0.215
Free Dox	0.745 ± 0.054	0.845 ± 0.058	0.548 ± 0.125

Notes: Data represent the mean ± SD (n=3). *statistical significance between PS5-DoxL and Free Dox; #statistical significance between PS5-DoxL and 5-Dox (* and # p <0.05)

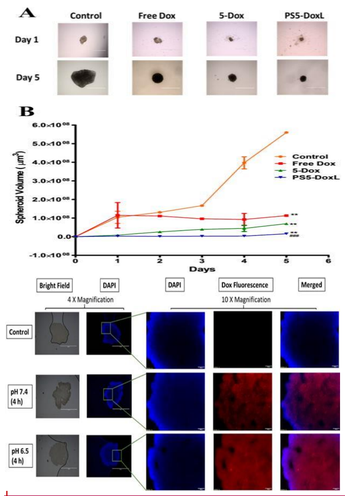

Fig 12. (A) Morphological images and (B) quantitative graph for inhibition of spheroid growth of BT474. Scale bars correspond to 1000 µm. Data presented as mean±SD (n=5).

Fig 13. BT474 3D-MCTS treated with PS5-DoxL for 4 h in pH 7.4, pH 6.5, or without treatment as control.

Conclusion

In summary, a pH-sensitive liposome formulation containing peptidomimetic doxorubicin conjugate was developed targeting HER2 positive lung and breast cancer cells. The PS5-DoxL formulation could significantly improve selectivity toward HER2 positive cancer cell lines and increase Dox accumulation in the tumor for better antitumor efficacy.