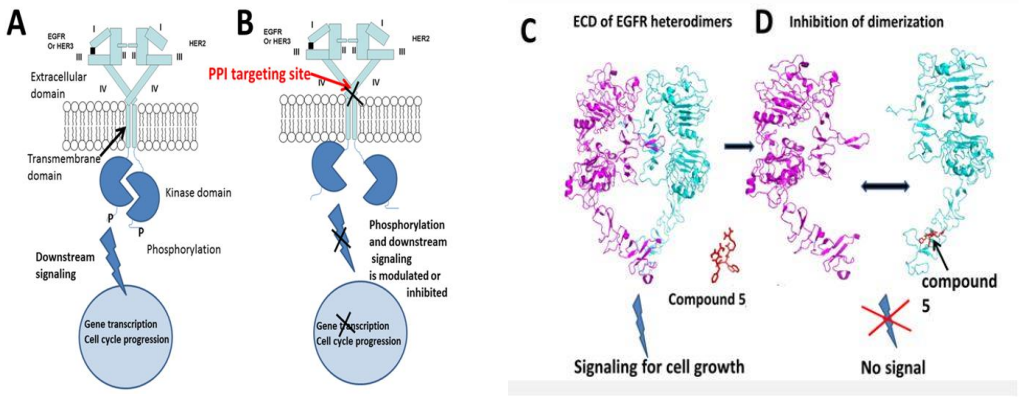
A pH-sensitive liposome formulation of peptidomimetic-doxorubicin conjugate for targeted delivery of anticancer conjugate on HER2 positive lung and breast cancer

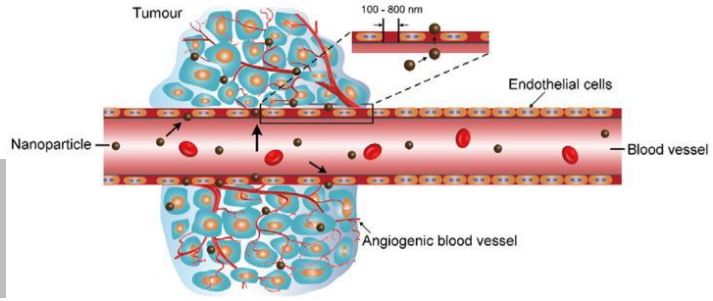
Jafrin Jobayer Sonju, Sitanshu S. Singh, Achyut Dahal, Seetharama D. Jois

Background

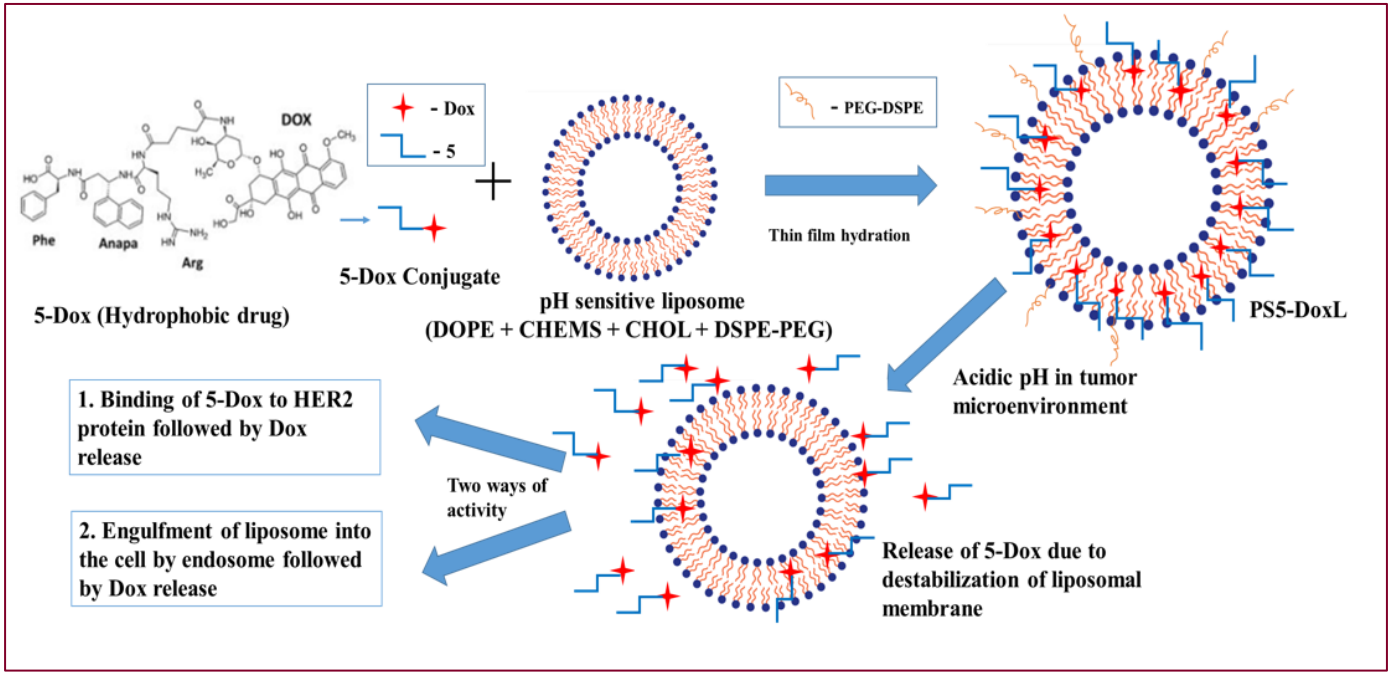
- Human epidermal growth factor receptors (EGFRs) have 4 extracellular domains (I- IV). Domain IV is near the transmembrane domain and is known to stabilize the protein-protein interaction (PPI) between human epidermal growth factor receptor 2 (HER2) and its dimerization partner (1).
- Overexpression of HER2 and HER2-HER3 dimerization is important in 30% of breast cancer (2) and EGFR and HER2 are overexpressed in 30% of non-small cell lung cancer (NSCLC) (3).
- Peptidomimetic compound 5 binds to the extracellular domain IV of HER2 and inhibits HER2 dimerization with other EGFR (4).



- Doxorubicin (Dox) is a well-known anticancer drug used in treating different types of cancers such as lung cancer, colon cancer, breast cancer, leukemia, etc (5).
- Dox lacks tumor-specific activity because it targets all dividing cells and, at higher doses, Dox treatment causes irreversible cardiotoxicity (6). To improve its selectivity and anticancer activity, Dox-compound 5 conjugate was prepared that specifically targets HER2-positive cancer cells but was limited in serum stability.
- Therefore, a pH-sensitive liposomal formulation (PS5-DoxL) containing Dox-compound 5 conjugate was prepared to cargo the compound while maintaining 5-Dox stability and after selective accumulation in the tumor microenvironment, release the 5-DOX conjugate in an acidic environment.



Design Strategy and Objectives



Objectives:

- This research focuses on the selective delivery of a peptidomimetic ligand attached Dox conjugate on the HER2 positive lung and breast cancer cells transported by a pH-dependent liposomal formulation system for the enhancement of targeted anticancer treatment.
- The liposomal formulation containing targeting ligand conjugated cytotoxic drug can be an effective approach to specifically deliver chemotherapeutic drugs to cancer cells that overexpress a particular cell surface receptor.

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	-

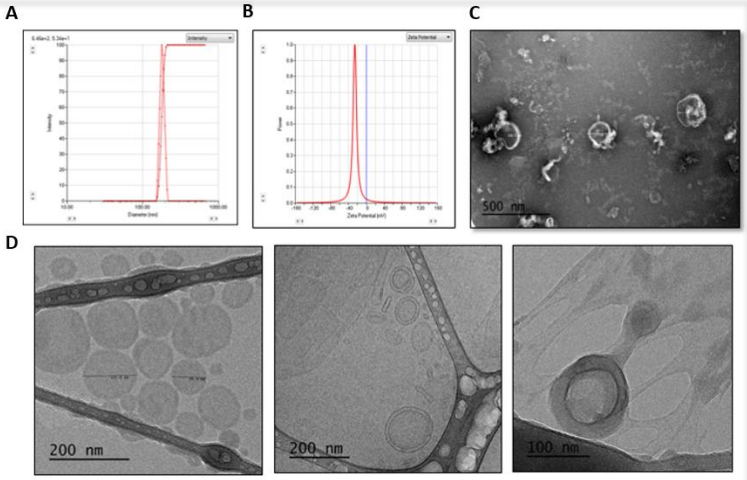


Fig 1. (A) Particle Size distribution and **(B)** Zeta Potential graphs of PS5-DoxL **(C)** TEM and **(D)** cryo-TEM images are showing the morphological characteristics of PS5-DoxL at different magnifications.

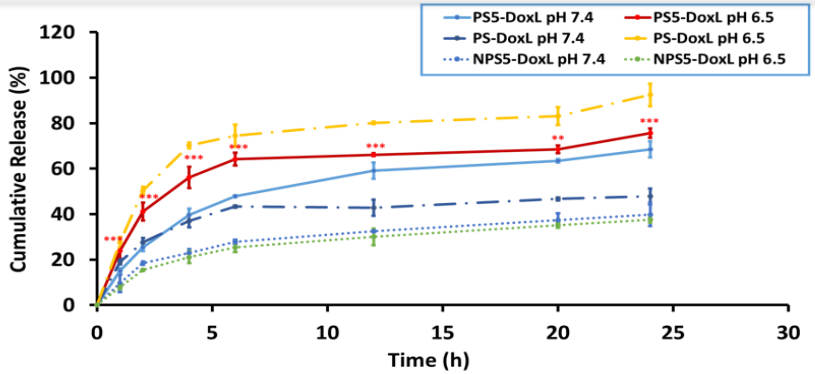


Fig 2. *In vitro* release of 5-Dox from PS5-DoxL and NPS5-DoxL or free Dox from PS-DoxL at 37 °C, pH 6.5 and 7.4 PBS buffer medium, respectively. Data represent the mean ± SD (n=3). Statistical significance between PS5-DoxL 7.4 and PS5-DoxL 6.5 (** p <0 .01 and *** p <0 .001).

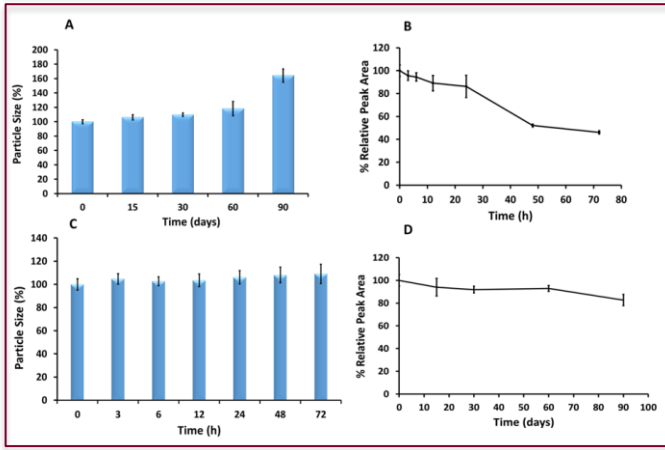


Fig 3. Stability data of **(A)** PS5-DoxL long term stability stored at 4º C up to 90 days analyzed in particle size analyzer, **(B)** 5-Dox in PS5-DoxL stored at 4º C up to 90 days analyzed in HPLC, **(C)** PS5-DoxL in human serum up to 72 h, and D. 5-Dox in PS5-DoxL in human serum up to 72 h analyzed in HPLC.

Results

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

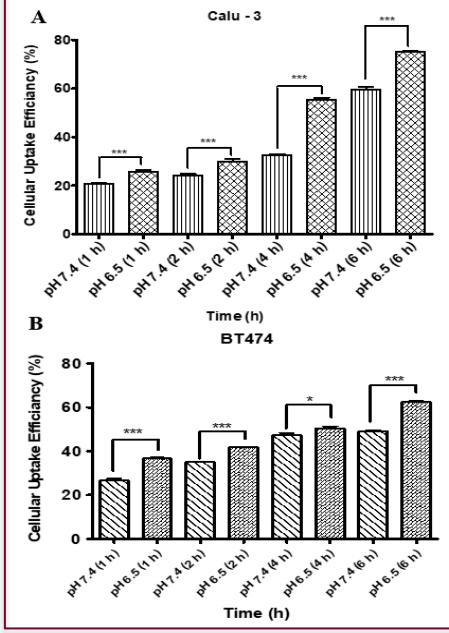


Fig 4. In vitro cellular uptake analysis by spectrofluorometer up to 6h in **(A)** Calu-3 and **(B)** BT474 cell lines treated with PS5-DoxL in pH 7.4 and pH 6.5.
Notes: Data represent the mean ± SD (n=3). Statistical significance between pH 7.4 and pH 6.5 (* p <0.05, ** p <0.01, and *** p <0 .001).

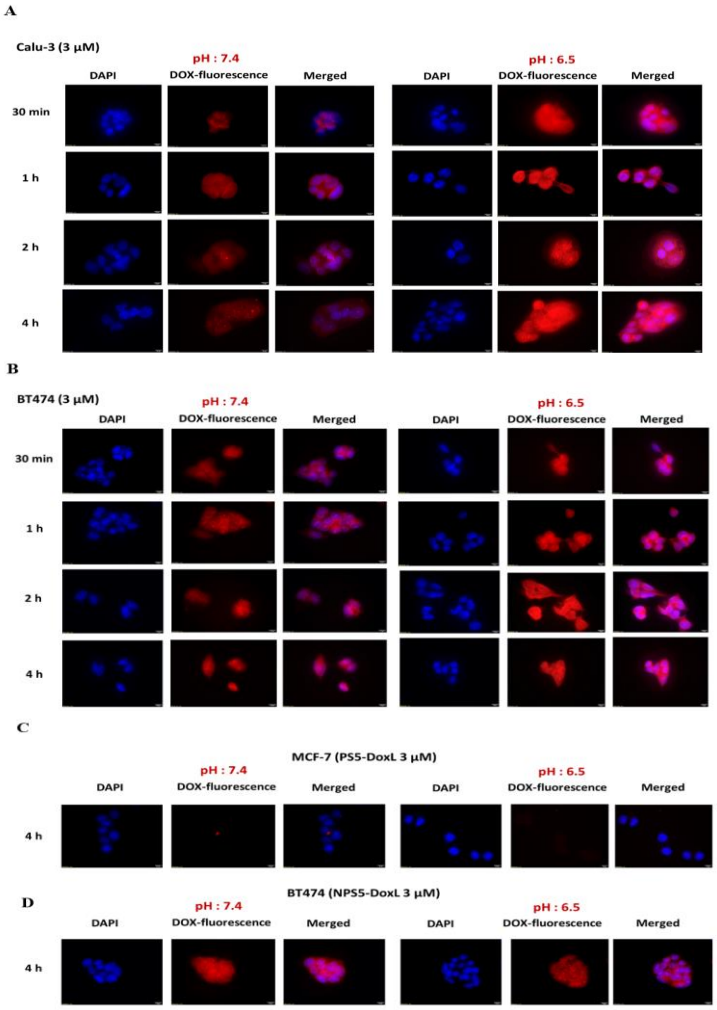


Fig 5. Time-dependent uptake of PS5-doxL in **(A)** Calu-3 and **(B)** BT474 cells in pH 7.4 and pH 6.5 for 30 min, 1 h, 2 h, and 4 h. **(C)** Uptake of NPS5-DoxL in BT474 cells in pH 7.4 and pH 6.5 for 2 h. (60x magnification). The scale bar corresponds to 10 µm.

Results

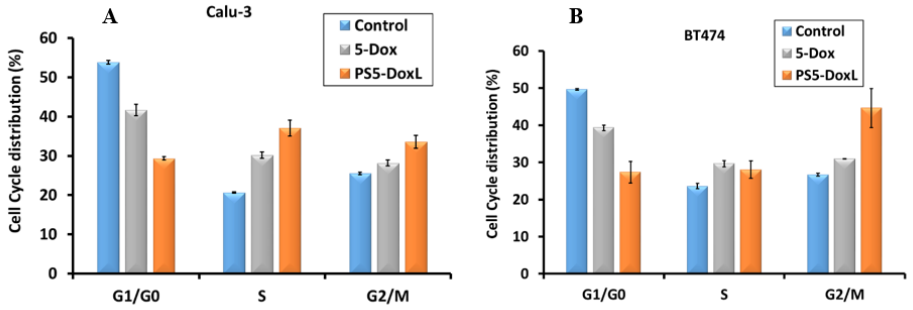


Fig. 6 Effect of 5-Dox and PS5-DoxL on cell cycle arrest in HER2-overexpressed cancer cells **(A)** Calu-3 and **(B)** BT474. Data represented as mean ± standard deviation.

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)

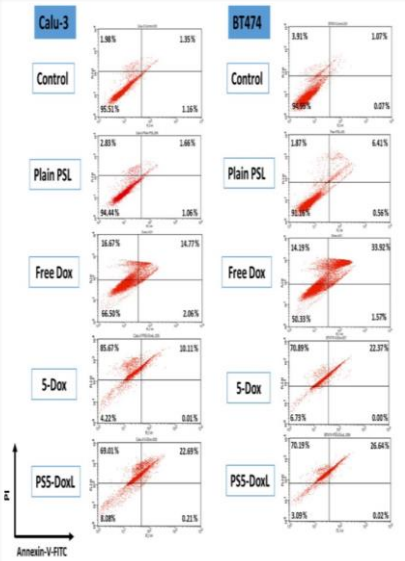


Fig. 7 Apoptosis study on Calu-3 and BT474 after treatment with 5-Dox, Free Dox, and PS5-DoxL for 12 h.

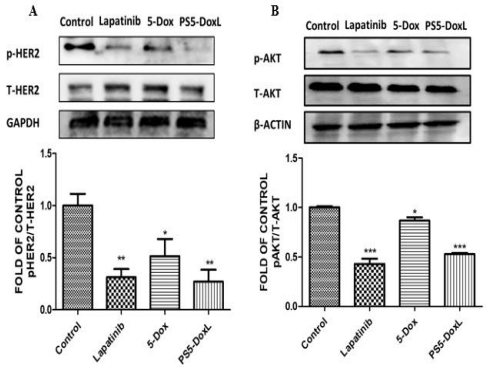


Fig 8. PS5-DoxL inhibits the phosphorylation of HER2 and Akt. Western blot analysis of **(A)** phosphorylation of HER2 and B. Akt after treatment of vehicle control, Lapatinib (1 µM), 5-Dox (3 µM), and PS5-DoxL (3 µM) for 36 h in Calu-3 cells. The visualization of A. GAPDH and **(B)** β-Actin was used to ensure equal sample loading in each lane. Data represented as mean ± standard deviation *p<0.05, **<0.01, and *** P<0.001.

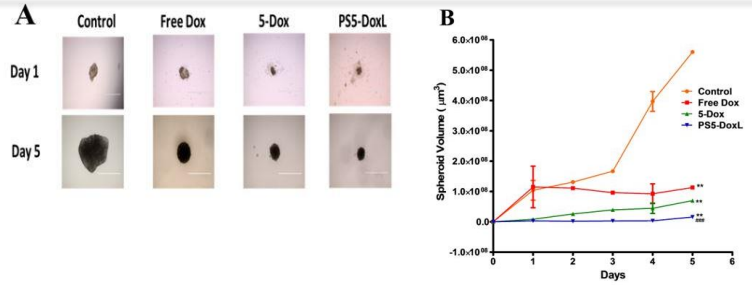


Fig 9. BT474 3D-MCTS treated with PS5-DoxL for 4 h in pH 7.4, pH 6.5, or without treatment as control.

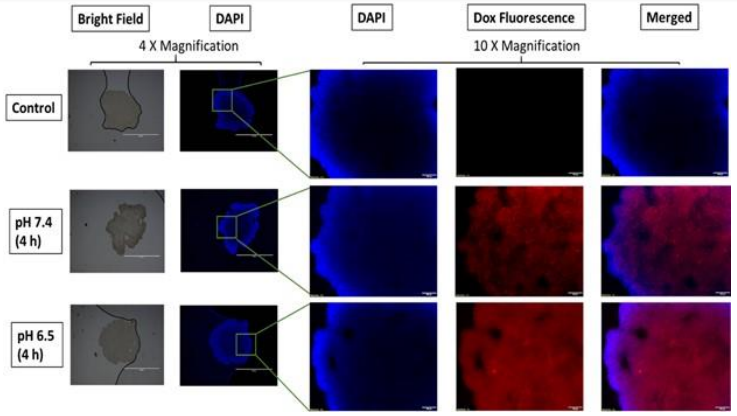


Fig 10. BT474 3D-MCTS treated with PS5-DoxL for 4 h in pH 7.4, pH 6.5, or without treatment as control.

Discussion

- The PS5-DoxL formulation showed optimum size for accumulation into the tumor microenvironment with PDI 0.20 indicating homogeneity of particles. Zeta potential also indicates stable dispersion of particles with less undesirable interaction with other serum proteins.
- In vitro release study indicated the pH-dependent release of 5-Dox in an acidic environment in tumor tissue which can reduce side effects.
- Antiproliferative activity indicated that PS5-DoxL showed higher efficacy and selectivity toward HER2 positive cancer cell lines compared to HER2 negative and noncancerous cell lines.
- The uptake studies indicated that PS5-DoxL had a significant pH-dependent release in pH 6.5 compared to pH 7.4 and can be evaluated within 30 min by fluorescence microscopic analysis.
- Cell cycle analysis and apoptotic studies revealed the cytotoxic effects of PS5-DoxL are congruent to the free Dox treatment and initiated cell cycle arrest and apoptotic cell death.
- Western blot results on Calu-3 cells which overexpress HER2 protein indicated that PS5-DoxL had activity in inhibition of HER2 protein and subsequent signaling.
- The PS5-DoxL formulation showed higher specificity toward HER2 positive lung and breast cancer cells supported by in vitro 3D-multicellular tumor spheroid (3D-MCTS) antiproliferative, antitumor assays. pH-dependent drug uptake was also showed higher uptake in pH 6.5 condition in the 3D-MCTS assay.

Conclusion

- In summary, a pH-sensitive liposome formulation containing peptidomimetic doxorubicin conjugate was developed targeting HER2 positive lung and breast cancer cells.
- To the best of our knowledge, the present study marks the first-time reporting of the stimuli-sensitive liposomal formulation of the doxorubicin-peptidomimetic conjugate in a targeted therapeutic approach.
- 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.

Thank you

References: 1.Yarden and Pines (2012) Nat Rev Cancer 12: 553-563.2.Lee-Hoeflich et al., (2008), Cancer Res. 68: 5878-873.Hofheinz et al., (2005) Anticancer Drugs;16 691-707.4.Satyanarayanajois S. et al., Chem Biol Drug Des. 2009; 74:246–257.5.Minotti G. et al., Pharmacol Rev. 2004;56:185–2296.Kwok JC et al., Mol Pharmacol. 2003; 63:849–861.7.Hofheinz R.D et al.,.Anticancer Drugs 2005;16 691-707