Supplementary information

Targeted co-delivery of aldose reductase inhibitor epalrestat and chemotherapeutic doxorubicin via redox-sensitive prodrug approach promotes synergistic tumor suppression

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2. Methods

2.1 TEM and AFM analysis

The Redox triggered changes in morphology was observed using transmission electron microscopy (TEM) and Atomic force microscopy (AFM). For TEM analysis, the formulations were prepared by negative staining procedure by administering one drop (10 µL) of formulation onto a copper grid and stained with 2.0% (w/v) phosphotungstic acid. After proper air drying for about 30 min, the samples were analyzed using a transmission electron microscope (TECNAI 200 Kv TEM, Fei, Electron Optics, USA).

Similarly, size and surface topography of the Dox/EPR-SS-TPGS and Dox/EPR-SS-TPGS-B6 micelles was analyzed by Atomic Force Microscopy (APE Research, Italy) in non-contact mode. 10 µL of diluted formulation was drop casted on a clear glass cover slip and air dried at ambient room temperature conditions. Non-contact mode cantilevers with corresponding properties were

used and scanning was performed at different scan areas to obtain clear pictures. Subsequent image processing was performed using Gwyddion 2.3 software to obtain the 3D surface topography of the samples.[1] In both the techniques, to observe the GSH triggered morphology changes, micelles were incubated in 10 mM DTT and difference was observed at 6 h post incubation.

2.2 HPLC detection of EPR and Dox

Analytical and bio analytical quantification of EPR and Dox was evaluated using RP-HPLC with PDA and fluorescent detectors (Shimadzu system equipped with ATvp binary gradient pumps). Same eluent and instrument conditions were maintained for both in vitro and in vivo samples. The collected plasma samples and harvested tumors were weighed and homogenized on ice. To the extracted 0.1 mL of plasma or tissue homogenate with 0.4 mL of acetonitrile solution and extracts were vortexed for 10 min and centrifuged at 10000 rpm for 10 min. The EPR and Dox content was analyzed in the supernatants using HPLC analysis and data acquisition was performed using LC solutions software. The mobile phase delivered at a flow rate of 1.00 mL/min, comprised of acetonitrile and acetate buffer (pH 3.8) (45: 54.75 v/v) containing 0.25% v/v of tetra methyl ammonium hydroxide as ion pairing agent. EPR was analyzed at 393 nm using PDA detector and Dox was analyzed using fluorescence detector at 470 nm and 550 nm excitation and emission wavelength respectively.

2.3 Measurement of critical micellar concentration (CMC)

The CMC of, EPR-SS-TPGS conjugate was determined by fluorescence method using pyrene as as a fluorescent probe. In brief, 1 ml pyrene solution (5 $x10^{-4}$ M) was dissolved in acetone and the evaporated under nitrogen flow. EPR-SS TPGS at different concentrations ranged from 1 -500 µg/mL were added into pyrene solution to attain 5 $x10^{-7}$ M final pyrene concentration and samples were subjected to untrasonication for 30 min and kept in dark at room temperature overnight. The

fluorescence intensity of samples were measured using spectro flourimeter (shimadzu RF-6000) with the excitation and emission spectrum ranging from 305 to 350 nm and the emission wavelength of 390 nm. The 334 nm (I_{334}) and 337 nm (I_{337}) intensity ratio of was recorded and analyzed for determination of CMC value.[2, 3]

3 Results

3.1 ^{1}H NMR of TPGS

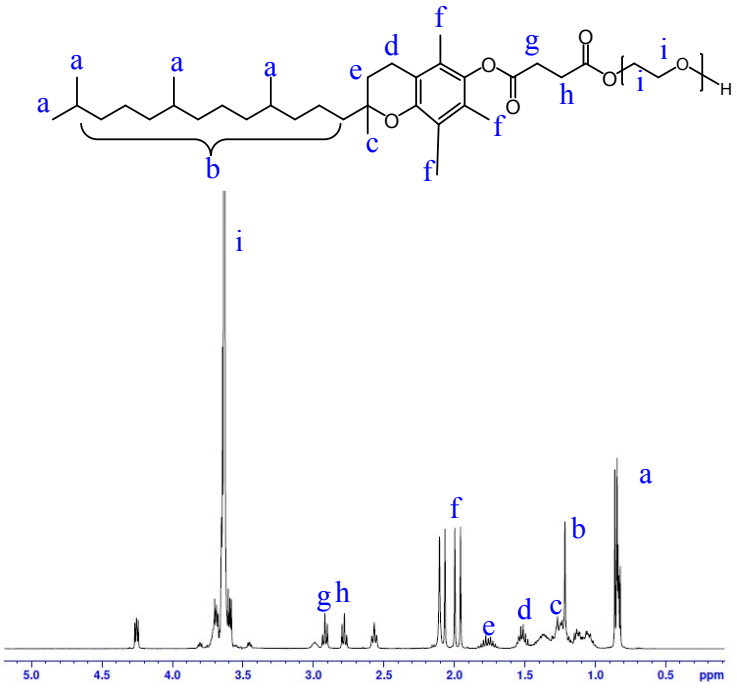

Figure S1: ^{1}H-NMR of TPGS

3.2 CMC determination

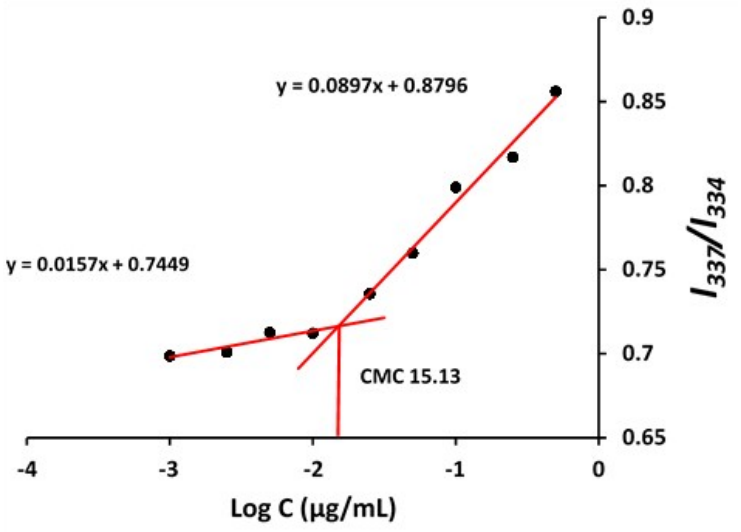

Figure S2: Critical micellar concentration (CMC) EPR-SS-TPGS micelles

3.3 In vitro cell line studies

3.3.1 Synergy identification between Dox and EPR

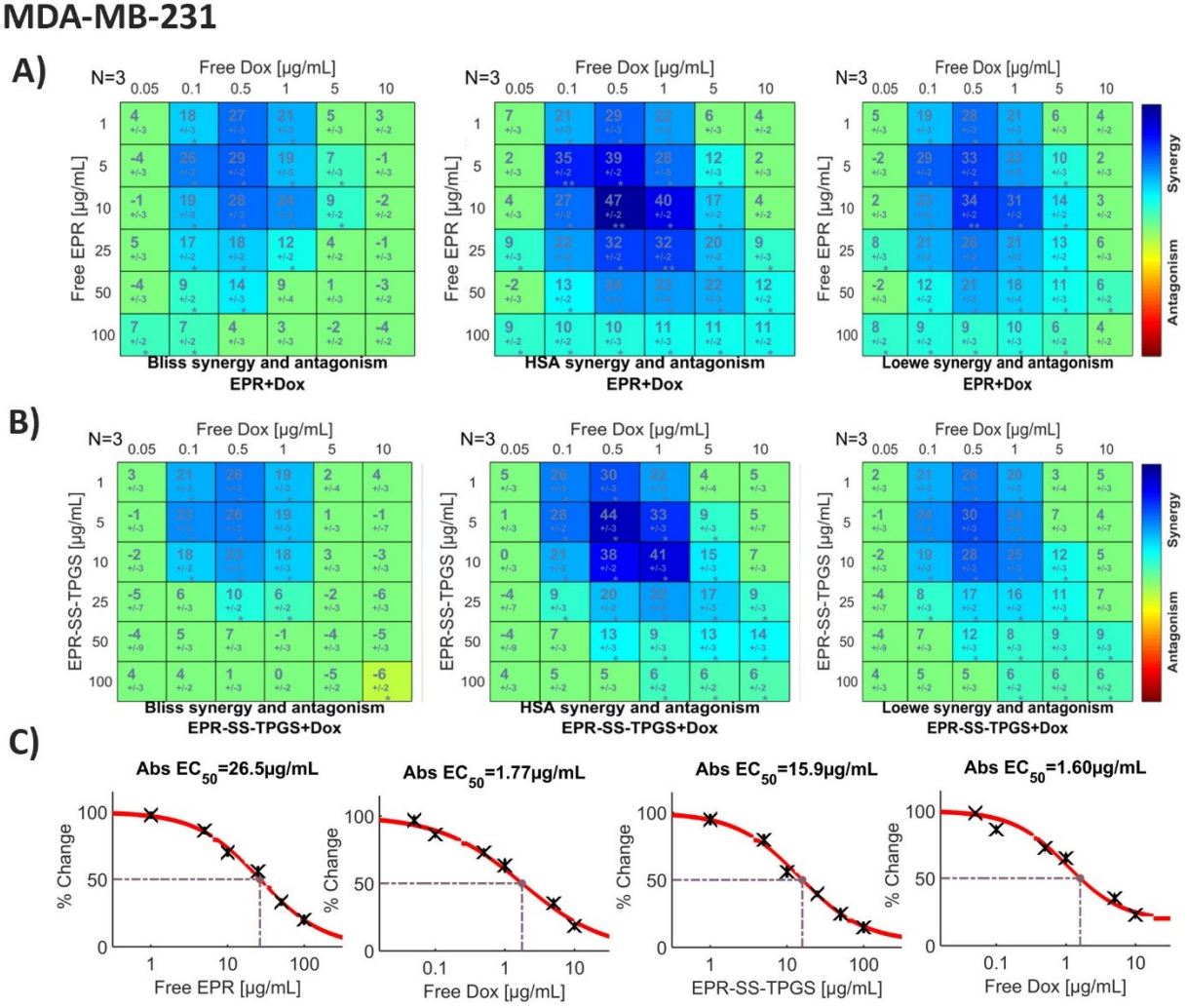

Figure S3: Predicted inhibition data of Bliss synergy model showing the region of synergy (blue spots) in MDA-MB -231 cells treated with A) free Dox (X-axis) and free EPR (Y-axis); B) free Dox (X-axis), and EPR-SS-TPGS (Y-axis) in 6 x 6 concentration checker board format for 48 h using MTT assay. C) IC50 values of free EPR, free Dox and EPR-SS-TPGS.

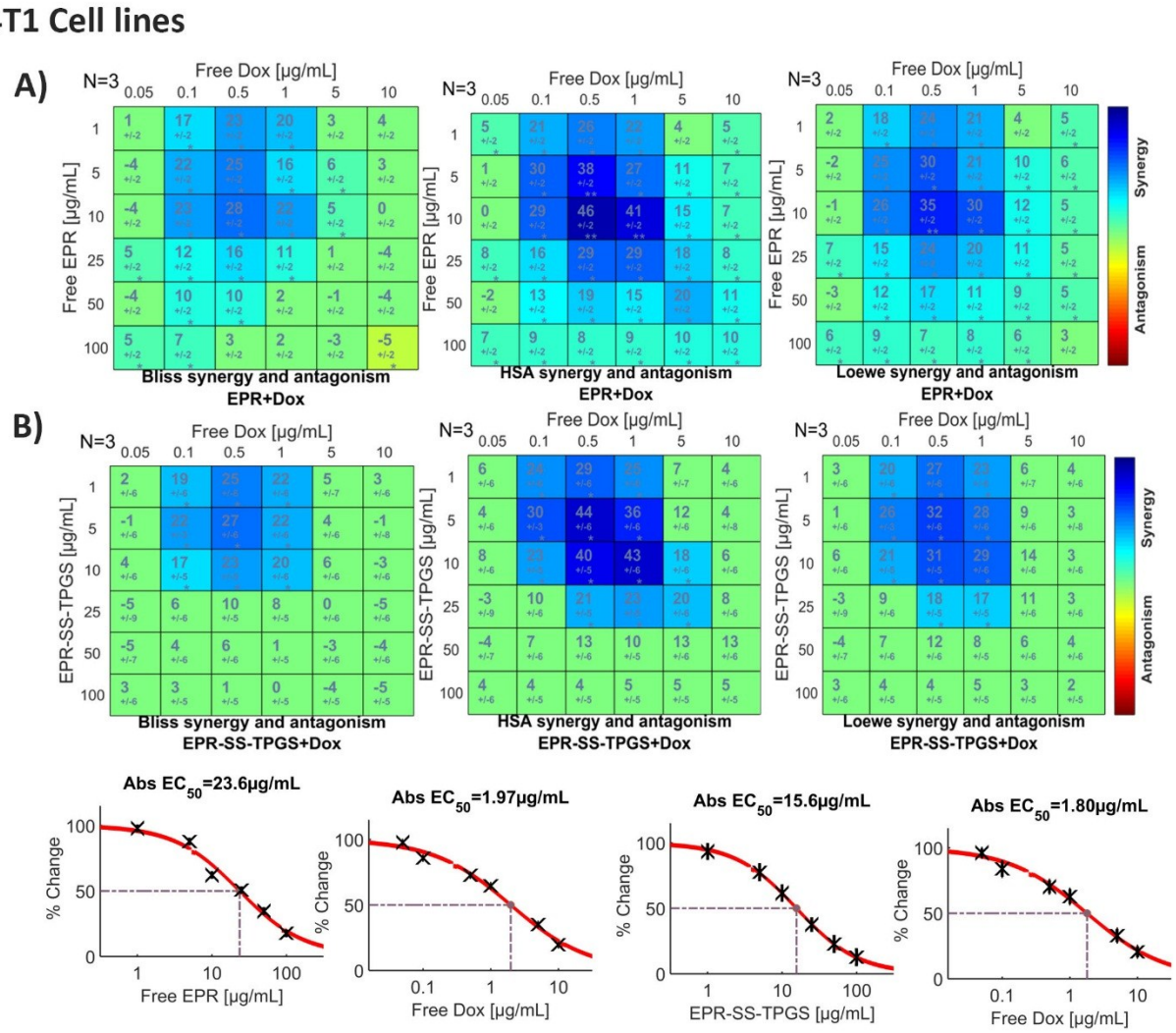

Figure S4: Predicted inhibition data of Bliss synergy model showing the region of synergy (blue spots) in 4T1.2 cells treated with A) free Dox (X-axis) and free EPR (Y-axis); B) free Dox (X-axis), and EPR-SS-TPGS (Y-axis) in 6 x 6 concentration checker board format for 48 h using MTT assay. C) IC50 values of free EPR, free Dox and EPR-SS-TPGS.

References

[1] Urandur S. Anisamide-Anchored Lyotropic Nano-Liquid Crystalline Particles with AIE Effect: A Smart Optical Beacon for Tumor Imaging and Therapy. 2018;10:12960-74.

[2] Zhang J, Zhao X, Chen Q, Yin X, Xin X, Li K, et al. Systematic evaluation of multifunctional paclitaxel-loaded polymeric mixed micelles as a potential anticancer remedy to overcome multidrug resistance. Acta Biomater. 2017;50:381-95.

[3] Wei H, Zhang XZ, Cheng H, Chen WQ, Cheng SX, Zhuo RX. Self-assembled thermo- and pH responsive micelles of poly(10-undecenoic acid-b-N-isopropylacrylamide) for drug delivery. Journal of controlled release : official journal of the Controlled Release Society. 2006;116:266-74.