Induction of mitochondrial cell death and reversal of anti-cancer drug resistance mediated via nanocarrier composed of triphenylphosphonium derivative of TPGS

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S1 Characterization of DTPP-TPGS

Size and surface charge (zeta potential) of DTPP-TPGS was measured using principles of photon correlation spectroscopy and electrophoretic mobility on a Zetasizer (3000HSA, Malvern UK). The instrument was calibrated with 5 nm standard polystyrene beads (Duke Scientific Corp., Palo Alto, CA) for particle size whereas DTS0050 was used for standardization of zeta potential. Size was measured in deionized water by recording relaxation times of light scattered by individual particulate entities of appropriately diluted DTPP-TPGS. Zeta potential was calculated using Smoluchowski approximation[1].

S1.1 Entrapment efficiency

Entrapment efficiency was determined by employing reverse phase liquid chromatography. Fixed volume of formulation was dissolved in methanol, and vortexed intensely for 5 min. The mixture was thereafter diluted with methanol again and analyzed via HPLC for entrapped drug content. A standalone LC-2010 (Shimadzu®, Japan) autosampler unit tagged with SPD-M10UV-PDA detector and a quarternary gradient pump was used as HPLC. DOX was analyzed on C-18 Purospher®STAR (250 mm × 4.6 mm, 5μm particle size) column using

a mobile phase of water:acetonitrile (65:35) with final pH adjusted to 3. Mobile phase was made to flow through the column at a rate of 1 mL/min and effluents were monitored at 234 nm.

S1.2 Electron microscopy

Shape and size of DTPP-TPGS was investigated using TEM. Briefly, diluted suspension of DTPP-TPGS was adsorbed onto carbon-coated film attached to a metallic grid. Excess sample was blotted off using filter paper and the grid was stained with uranyl acetate solution (1.5% w/v). The grid was air dried and sample was examined under transmission electron microscope at several magnifications (TECHNAI G^2 20 S-TWINFEI, Netherlands)[2]. For AFM imaging, DTPP-TPGS were collected on freshly cleaved mica substrate and allowed to air dry in form of a thin film. Thereafter surface characteristics were visualized by mounting on an AFM scanner (NT-MDT, Moscow, Russia) and probed in semi-contact mode at frequency of 120 kHz and a spring constant of 5-10 N/m[3].

S1.3 *In vitro* dissolution study

Dissolution study for DTPP-TPGS was carried out in PBS, pH 7.4, employing dialysis membrane method. DTPP-TPGS and equivalent DOX solution were filled in hermitically sealed dialysis bags and immersed in separate beakers containing PBS at 37±2 °C. Dissolution medium was stirred at 100 r.p.m. Aliquots of dissolution media containing dissolved drug were drawn at predetermined time points with fresh dissolution media added back at each sampling point to maintain sink conditions. Amount of drug released was analysed by reverse phase HPLC.

S2 LC-MS detection of DOX

LC-MS was set up as follows: LC-20AD Shimadzu UFLC pump with DGU-20A3 degasser, an SIL-HTc auto-sampler with a temperature-regulated pelteir-tray, a triple quadrupole API

4000 Q trap mass spectrometer (Applied Biosystems, Toronto, Canada), an Agilent Zorbex SB-Cyano column (3.5 μm, 100 x 4.6 mm), a guard column. Analysis was conducted under isocratic flow with mobile phase consisting of 30:70 (v/v) ammonium acetate (0.01M) acetonitrile blend passed through column for a total run time of 3 min.

S3 *In vivo* fluorescent imaging

To substantiate pharmacokinetic data, we conducted a supplementary *in vivo* imaging study in 4TI cell induced breast cancer carrying Balb/C mice on an IVIS® Spectrum (Caliper Life Sciences/ PerkinElmer) to generate pertinent fluorescent images after intravenous administration of DOX solution and DTPP-TPGS. Non-invasive whole body images were generated by subjecting treated animals to excitation radiation of 535nm. Subsequently generated emission was recorded at 560 nm. Images were acquired for 30 seconds at 5 min, 6 hours and 24 hours post-dosing and photon emission emanating from mice was analysed using Living Image® Software 4.4. In order to facilitate accurate imaging, mice were pre-anaesthetized using ketamine (10 mg/kg i.p.).

S4 NMR spectrum

(3-Carboxypropyl)triphenylphosphonium bromide (Purchesed from Sigma aldrich (1a)**)**: White solid; ^{1}H NMR (500 MHz, DMSO) δ = 7.93-7.75 (m, 15H, Benzene protons), 3.65-3.57 (m, 2H, P-CH_2), 2.50-2.47 (m, 2H, -**CH_2**-COOH), 1.78-1.70 (m, 2H, -CH_2-**CH_2**-COOH).

TPGS (D-α-Tocopherol polyethylene glycol-1000 succinate: Purchesed from Sigma aldrich (2a)**)**: White solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 4.29 (t, *J* = **4.67** Hz, 2H, CH_2), 3.64-3.62 (m, 44H, PEG), 2.92 (t , J = 2H, OCO-**CH_2**-), 2.80-2.76 (m, 2H, Ph-**CH_2**-), 2.57 (t, J = 2H, **CH_2**-COO-), 2.06, (s, 3H, **CH_3**), 1.99, (s, 3H, **CH_3**), 1.95, (s, 3H, **CH_3**),

1.90−1.70 (m, 2H, **CH2**), 1.60−1.00 (m, 24H, **CH**, **CH2**, **CH3**), 0.86 (d, 6H, 3J = 6.5 Hz, CH3), 0.82 (d, 6H, 3J = 6.5 Hz, CH3). Refer to figure S1**.**

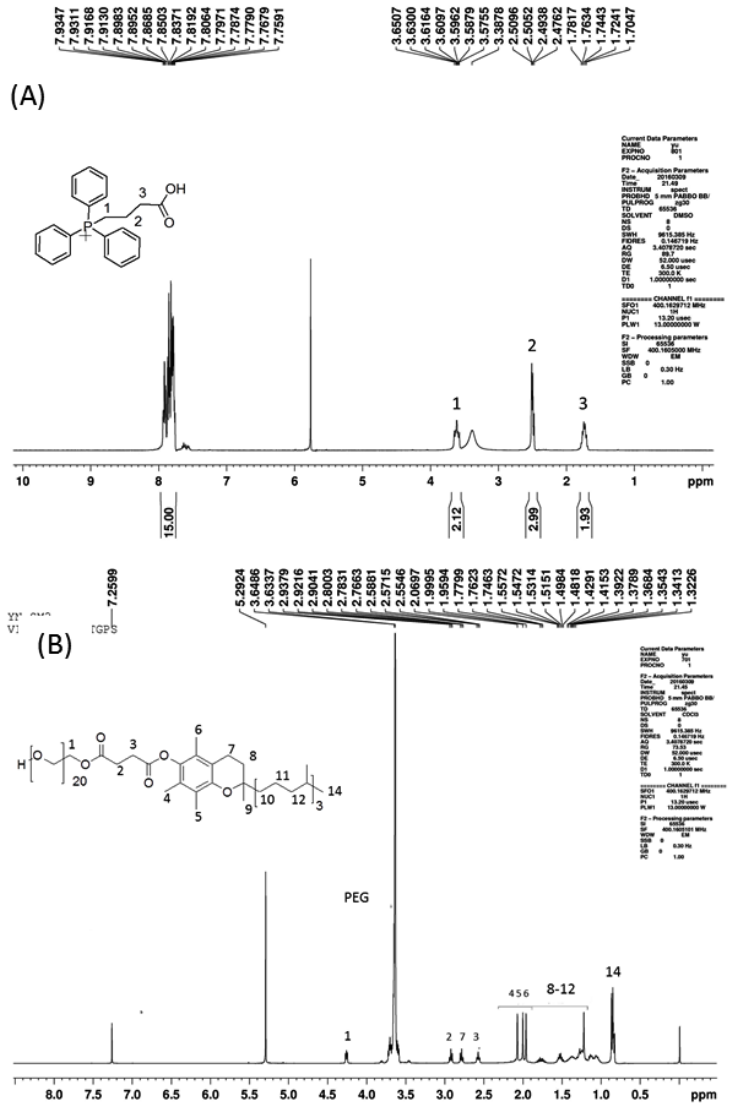

Figure S1: 1H NMR spectrum of (A) TPP, (B) TPGS

TPP-TPGS (D-α-Tocopherol polyethylene glycol-1000 succinate+(3-Carboxypropyl)triphenylphosphonium bromide conjugate: (3a)**)**: White solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.92-7.70 (m, 15H, **Benzene protons**), 4.29 (t, *J* = **4.67** Hz, 2H, CH_2), 3.64-3.65 (m, 46H, PEG+ P-CH_2), 2.92 (t , J = 2H, CH_2OCO), -2.80-2.76 (m, 2H, **C**H_2-COO) 2.57 (t, J = 2H, **C**H_2-COO-), 2.09, (s, 3H, **C**H_3), 2.02 (s, 3H, **C**H_3), 1.98, (s, 3H,

CH₃), 1.90−1.70 (m, 2H, **CH2**), 1.60−1.00 (m, 24H, **CH**, **CH2**, **CH3**), 0.86 (d, 6H, 3J = 6.5 Hz, CH3), 0.82 (d, 6H, 3J = 6.5 Hz, CH3). NMR spectrum is given in Figure S2.

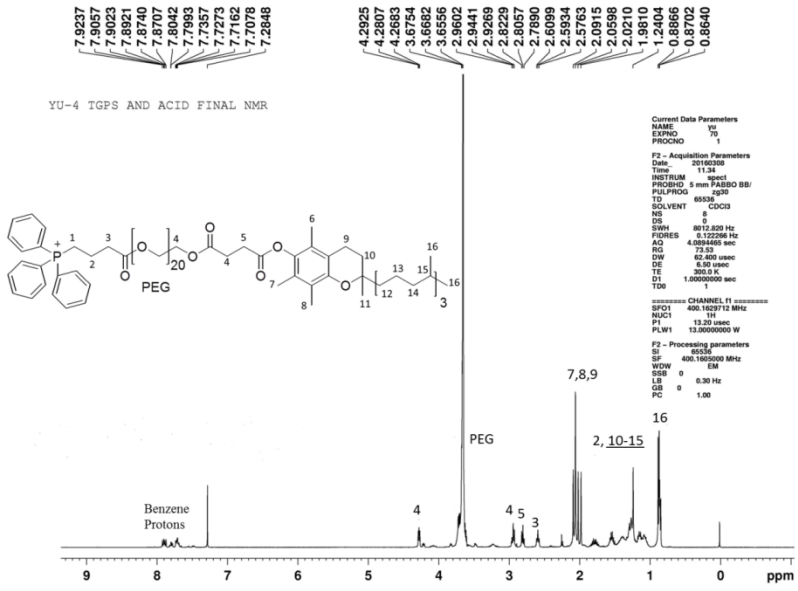

Figure S2: 1H NMR spectrum of TPP-TPGS

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