Supporting Information

Combination Therapy of NSCLC Using Hsp90 Inhibitor and Doxorubicin Carrying Functional Nanoceria

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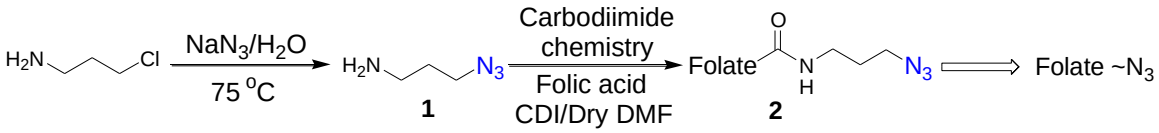
1. Preparation of folate~N_3 using carbodiimide chemistry.



Scheme S1. Preparation of azido-folate (**2**) from chloropropyl amine

Synthesis of Aminopropylazide: In a 100 mL round bottom flask, chloropropyl amine (7.0 g, 75.26 mmol) and sodium azide (14.23 g, 225.81 mmol) were added to 40 mL of distilled water and heated at 80 ^{o}C for 20 h. The reaction mixture was concentrated in a rotary evaporator at high vacuum, 2 g of KOH were added and the product was extracted using diethyl ether. Subsequently, the reaction mixture was dried over anhydrous sodium sulphate and concentrated. Finally, the product was purified via flash column chromatography using 4% ethyl acetate in petrolium ether as an eluant. Yield: 5.1 g (68%). ^{1}H NMR (300 MHz, $CDCl_3$, d ppm): 1.26 (bs, 2H), 1.81 (m, 2H), 2.80 (t, 2H), 3.38 (t, 2H). FT-IR (CHCl3): 3307, 2941, 2089, 1663, 1433, 1370, 1259, 1242, 1075, 1026, 818, 760 cm^{-1}.

Synthesis of azide-functionalized folic acid: To a solution of folic acid (0.05 g, 0.12 mmol) in DMSO (2 mL), EDC (0.021 g, 0.11 mmol) and NHS (0.013 g, 0.11 mmol) in 0.5 mL MES buffer (pH =5.0) were added and then incubated at room temperature for 3 minutes. To this resulting reaction mixture was added drop-wise ethylenediamine (0.007 g, 0.11 mmol) in 0.25 mL of PBS (pH = 7.4) and then incubated for 3 h at room temperature. The reaction mixture was centrifuged and washed to remove excess starting materials. The azide-functionalized folic acid was dissolved in 1 mL of DMF until further use. Yield: 0.05 g (86%). The presence of a band at

2097 cm^{-1} in the IR spectrum and a UV absorbance shoulder at 354 nm confirmed the formation of azide-functionalized folic acid. ^{1}H NMR (300 MHz, DMSO-d_6, d ppm): 1.61 (m, 2H), 1.65 (m, 2H), 1.90 (m, 2H), 2.19 (t, 2H), 2.78 (t, 2H), 4.18 (q, 1H), 4.21 (d, 2H), 6.62 (d, 2H), 7.59 (d, 2H), 8.58 (s, 1H). FT-IR (Neat): 3024, 2097, 1685, 1603, 1492, 1375, 1291, 1248, 1180, 1122, 1062, 950, 844, 755, 696 cm^{-1}.

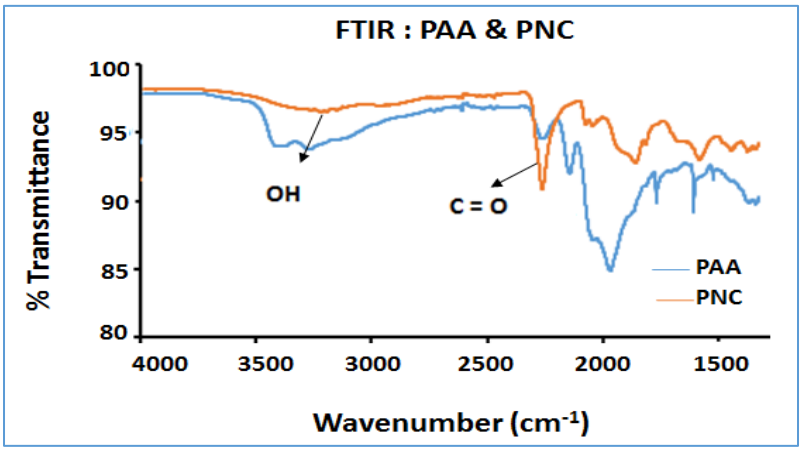


Figure S1. Confirmation of the presence of polyacrylic acid coating on the surface of nanoceria by using FT-IR spectroscopy. The presence of a band at 1710 cm^{-1} in the FT-IR spectra of PAA-NC preparation (orange) when compared with pure PAA polymer (blue), indicated the presence of PAA-coatings on nanoceria.

DLS-size and surface charge on NCs

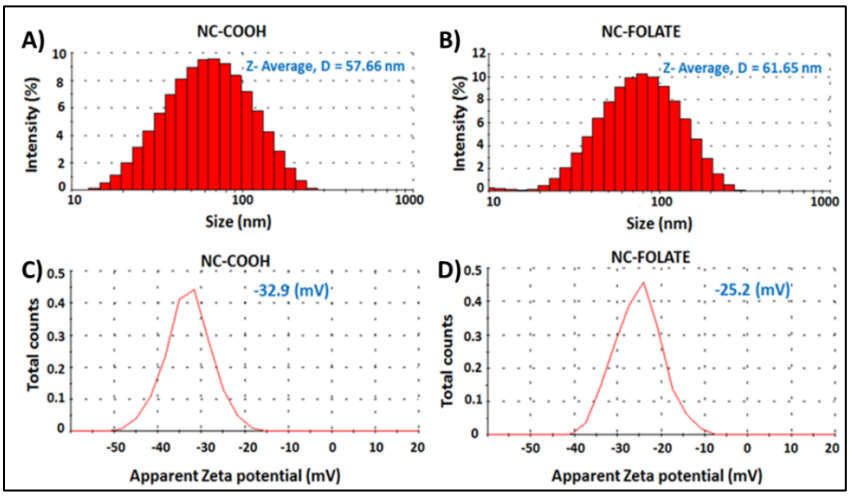


Figure S2. Dynamic light scattering experiments resulted in achieving desirable monodispersed stable nanoceria with an average diameter of **A**) 57.66 nm for PNC and **B**) 61.65 nm for FNC. The overall surface charge of PNC and FNC were characterized by zeta potential measurements, and were found to be **C**) ζ= -32.9 mV and **D**) ζ= -25.2 mV, respectively.

Internalization of FNC in the presence of free folate

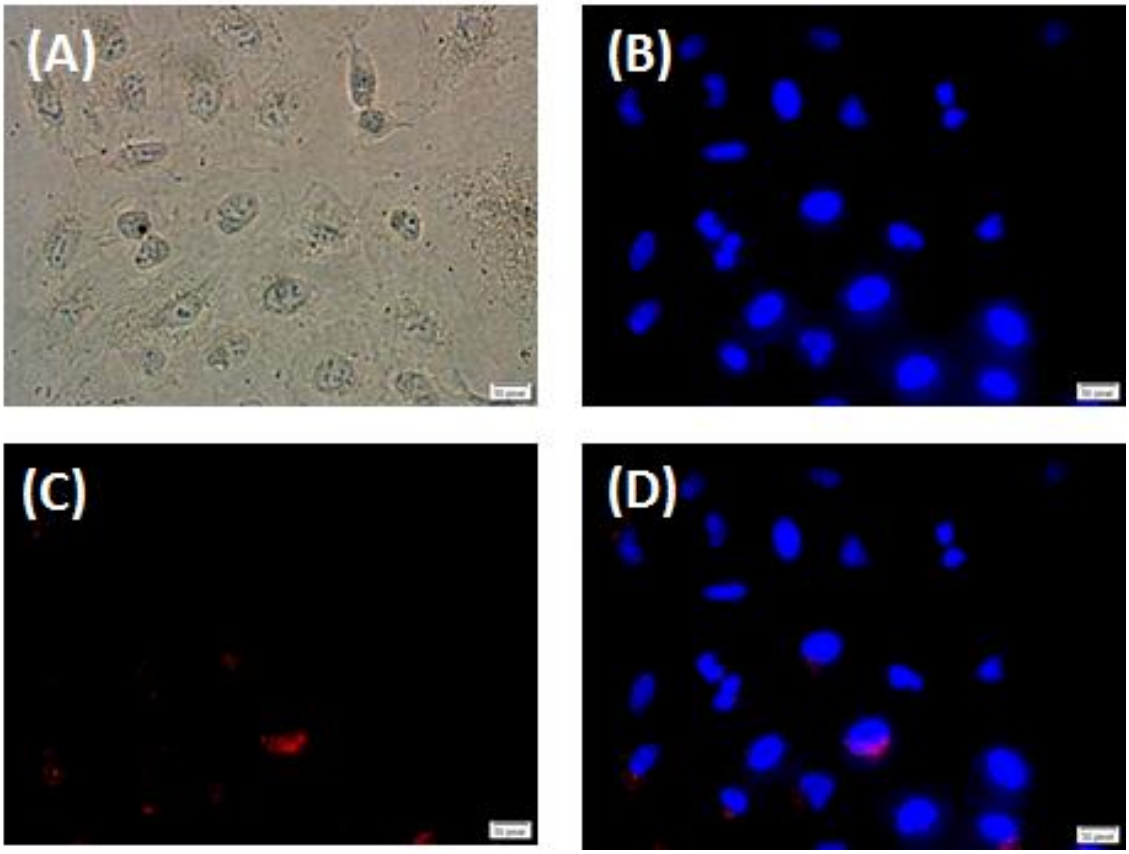


Figure S3. Determination of internalization mechanism. The A549 cells were pre-incubated for 6 h with excess of free folic acid prior to incubation with functional FNC. Fluorescence microscopic images showed minimal internalization into A549 cells, indicated for the folate-receptor mediated internalization of nanoceria.

Quantitative analysis of apoptosis and necrosis assays

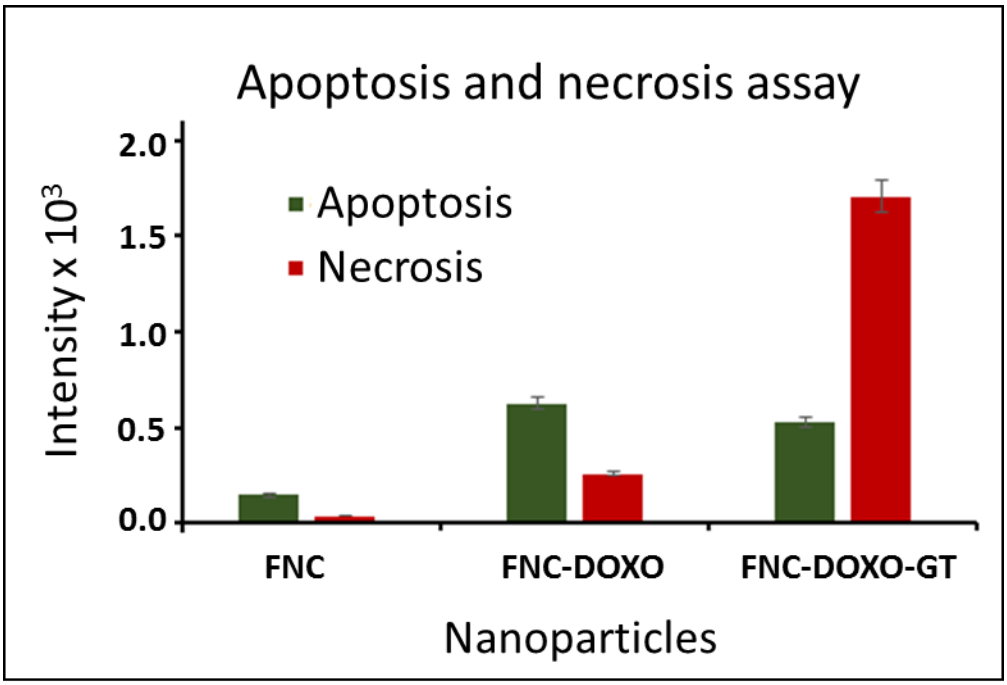


Figure S4: Quantitative analysis of apoptosis and necrosis assays using IMAGE J software. Results showed higher apoptosis from FNC-Doxo and higher necrotic events when FNC-Doxo-GT are used. Average values of three measurements are depicted ± standard error.